Bulletin No. 19 of the Department of Surgery,
University of Lund, S-221 85 LUND,
1979.

PANCREATIC ENZYME SECRETION

The influence of intraduodenal bile-pancreatic juice components

PER LILJA

Lund 1979

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41553413_0021

PANCREATIC ENZYME SECRETION.

The influence of intraduodenal bile-pancreatic juice components.

Per Lilja
med. lic., Ld.

Akademisk avhandling

som för avläggande av medicine doktorsexamen
vid medicinska fakulteten vid Universitetet i Lund
kommer att offentligen försvaras å Föreläsningssal 3,
Centralblocket, Lasarettet i Lund,
fredagen den 14 dec 1979 kl 13.00

Organisation
LUND UNIVERSITY
Department of Surgery
University of Lund
S-221 85 LUND
Sweden

Document name
DOCTORAL DISSERTATION
Date of issue
1979-12-14
CODEN: LUMEDW/(MESL-1002)/1-109/(1979)

Author(s)
Per Lilja

Sponsoring organisation

Title and subtitle

PANCREATIC ENZYME SECRETION.
The influence of intraduodenal bile-pancreatic juice components

Abstract

The influence of bile-pancreatic juice components on the pancreatic enzyme secretion in rat, pig, and man was investigated. Exclusion of (bile-)pancreatic juice from the duodenum resulted in a hypersecretion of pancreatic enzymes, which was reversed by reinfusion of the juice. Intraduodenal administration of trypsin inhibitor in the presence of (bile-)pancreatic juice enhanced the release of pancreatic enzymes. In rat, these secretory changes were characterized by a parallel increase and a parallel decrease, respectively, of the output of amylase, lipase, and trypsinogen. Trypsin inhibitor per se was without effect. Intraduodenal infusion of amylase (rat, pig) or lipase (rat) did not influence the pancreatic enzyme secretion, which, however, was markedly depressed by trypsin infusion (all species); additional infusion of bile did not influence this depression (rat). Vagotomy or cholinergic blockade abolished the trypsin-induced changes of the pancreatic secretion in the rat. Vagal stimulation by insulin-induced hypoglycaemia failed further to enhance the protein output in rats, in which the bile-pancreatic juice was deviated from the intestine. Intraduodenal infusion of trypsin during insulin-induced hypoglycaemia, however, markedly inhibited the protein secretion. Pancreatic supplements, when administered as granulated pancreatin, caused an increase of the activities of digestive pancreatic enzymes in the upper part of the gut in patients with pancreatic insufficiency.

The results show that intraluminal trypsin - in contrast to amylase or lipase - - exerts a feedback control of pancreatic enzyme secretion in rat, pig, and man. Intraduodenal bile does not affect this control mechanism; intact vagal function is, however, essential. Granulated pancreatin may induce changes in the intestinal pancreatic enzyme activities great enough to interfere with the observed feedback mechanism.

Key words
Amylase, Bile, Cholinergic blockade, Feedback, Intraduodenal, Lipase, Pancreatic enzyme secretion, Trypsin, Vagal stimulation, Vagotomy.

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language
English

ISSN and key title

ISBN

Recipient's notes

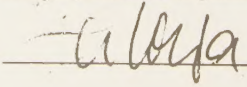
Number of pages
109

Price

Security classification

Distribution by (name and address)
Per Lilja, Department of Surgery, University of Lund, S-221 85 Lund, Sweden.

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature  Date 1979-12-14

DOKUMENTDATABLAD enl SIS 61 41 21

From the Department of Surgery and the Department of Pharmacology,

University of Lund, Lund, Sweden

PANCREATIC ENZYME SECRETION

The influence of intraduodenal bile-pancreatic juice components

by

Per Lilja

Lund 1979

EFFECTS OF INTRADUODENAL AMYLASE, LIPASE, TRYPSIN AND BILE ON PANCREATIC ENZYME SECRETION IN RAT

Per Lilja

Department of Surgery, University of Lund,

S-221 85 Lund, Sweden

Abstract

The effect of duodenal infusion of trypsin, amylase, lipase and bile on pancreatic enzyme secretion was studied in conscious rats surgically prepared with bile-pancreatic fistulae. Trypsin infusion resulted in a depression of the secretory volume and protein and trypsinogen output. All these effects were reversed after additional infusion of trypsin inhibitor. Infusion of amylase or lipase in doses equivalent to those of trypsin did not influence secretory volume, protein or enzyme output. Likewise intraduodenal bile infusion to rats with diverted bile-pancreatic juice did not change these parameters; also in rats with trypsin reinfused into the duodenum bile infusion was without effect.

Key-words: Amylase; Bile; Intraduodenal infusion; Lipase; Pancreatic secretion; Trypsin

Reprint requests: Per Lilja, M.D.
Department of Surgery
University of Lund
S-221 85 LUND
Sweden

INTRODUCTION

Ingestion of trypsin inhibitors by some rodent and avian species causes enlargement of the pancreas and increase of its digestive enzyme content (1, 4). These observations point to a relationship between intraduodenal tryptic activity and pancreatic exocrine function. A feed back regulation of pancreatic enzyme secretion exerted by intraduodenal trypsin has been shown based on findings in acute rat experiments (11, 15).

Macri et al. (17) recently reported pancreatic hypertrophy and increased amylase activity in pancreatic tissue in chicken fed albumin amylase inhibitors for 4 weeks. In acute experiments in pig we, however, did not find any influences on pancreatic secretion by intraduodenally administered amylase (13). Whether or not intraduodenal amylase affects pancreatic secretion in other species is not known; likewise the effect of lipase remains to be elucidated.

O'Donnel et al. (5) found an increase in the digestive enzyme concentrations of the pancreas but no influence on pancreatic weight after long-term oral administration of chenodeoxycholic acid to mice. The effect of intraduodenal administration of bile (or bile acids) on pancreatic secretion has been studied by several authors in different species (10, 18, 20, 22). The results reported are, however, contradictory.

A series of experiments was performed in which the pancreatic enzyme secretion was studied following duodenal administration of amylase, lipase and bile by comparison with trypsin in conscious rats surgically prepared with bile-pancreatic duct fistulae.

MATERIAL AND METHODS

Surgical procedure

Rats (Wistar; male) weighing between 250 and 300 g were used. Only one experiment was performed on each rat. Food and water was withdrawn from the animals immediately before the surgical procedures. The rats were anesthetized with ether and an abdominal incision was made exposing the bile-pancreatic duct and the duodenum. A polyethylene catheter with an inner diameter of 0.58 mm and a length of 15 cm (Intramedic PE-50) was introduced into the bile-pancreatic duct where it enters the intestine. Another catheter was inserted into the duodenum 5 mm below pylorus to permit direct duodenal administration. The animals were maintained in restraining cages and allowed 15 hours of postoperative recovery. During this period food was withdrawn from the animals but they had free access to fluid (1 part of Ringer's solution and 1 part of 5.5% glucose solution) until the start of the experiment. Animals in which surgical complications occurred were excluded from the study.

Intestinal infusion substances

Highly purified crystalline amylase (Sigma; Type II-A from Bacillus

Subtilis; 4 x crystallized) was dissolved in 0.05 M $NaHCO_3$. The activity of the solution was 100 000 μkat/l as determined by the Phadebas method (3). Porcine pancreatic lipase purified according to the method described by Donnér (6) was a gift from Dr Charlotte Erlanson, University of Lund, Sweden. The lipase was dissolved in 0.05 M $NaHCO_3$ in an amount giving an activity of 250-300 units/ml as analyzed with tributyrine as the substrate (8). Procine crystalline trypsin (A/S Novo, Copenhagen, Denmark) was dissolved in 0.05 M $NaHCO_3$ (2.5 mg/ml) corresponding to a tryptic activity of 350 U/ml as analyzed with TAME (p-toluene-sulphonyl-L-arginine methyl ester) as the substrate (12). Highly purified bovine lung trypsin inhibitor was obtained from Bayer AG, Leverkusen, Germany. The inhibitor is not absorbed from the intestine (23) or digested by intestinal proteolytic enzymes (9). In ancillary experiments it was found that 0.5 mg of the inhibitor completely abolished the tryptic activity of 1 mg of the trypsin used. Bile used for infusion was collected on ice from rats provided with bile duct fistulae and immediately frozen at -20°C the day before used in experiments. In ancillary experiments the bile was found to be free from tryptic (trypsinogen) activity.

The pH of the infusates was corrected to 8.0 ± 0.2. The solutions were kept on ice and the temperature brought to +25°C immediately before the introduction into the duodenum. The infusates were administered through the duodenal fistulae via an infusion pump with a constant rate of 1.2 ml/h which roughly corresponded to the volume spontaneously secreted from the bile-pancreatic duct.

Evaluation of pancreatic secretion

The bile-pancreatic secretion was collected in small tubes on ice in 30 minute intervals. The samples were immediately frozen at -20°C and analyzed in duplicate within two weeks.

The volume of the bile-pancreatic juice was estimated by weight and the concentration of protein according to the method described by Lowry et al. (16), as modified by Eggstein and Creutz (7). Lipase was assayed by a scaled down version of the method described by Erlanson and Borgström using tributyrine as the substrate (8). Amylase was determined according to the Phadebas test, a gift from AB Pharmacia, Uppsala, Sweden (3). Trypsinogen was estimated by activation of zymogens in bile-pancreatic juice by enterokinase (Miles Laboratories) as previously described (15). The tryptic activity was estimated by a modified Hummel method using TAME (p-toluene-sulphonyl-L-arginine methyl ester) as the substrate (12).

Calculations

The data obtained were subjected to statistical analysis using the Student's *t*-test. All values are given as mean ± SEM. Differences were considered significant at p-values below 0.05.

RESULTS

Effect of intraduodenal infusion of amylase on pancreatic enzyme secretion

Intestinal infusion (1.2 ml/h) of amylase (100 000 µkat/l 0.05 M $NaHCO_3$) in a dose calculated to correspond to double the amount of pancreatic amylase spontaneously secreted did neither change secretory volume nor protein or amylase output as compared to the spontaneous pancreatic secretion (Fig. 1).

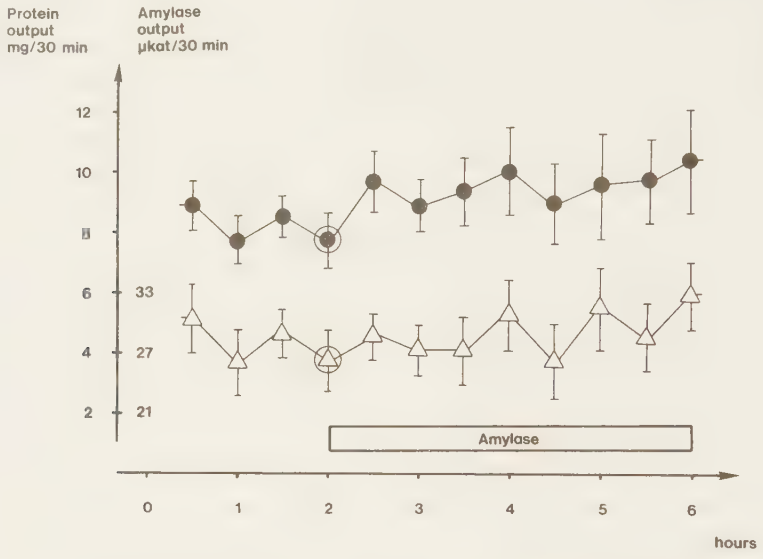


Fig. 1 Influence of duodenal infusion of amylase on protein (●—●) and amylase (△—△) output in rats provided with bile-pancreatic fistulae (n = 8). Values are given as mean ± SEM. The circles indicate the preinfusion values.

Effect of intraduodenal infusion of lipase on pancreatic enzyme secretion

Intestinal infusion (1.2 ml/h) of purified pancreatic lipase free of co-lipase activity in a dose calculated to correspond to double the amount of lipase spontaneously secreted (650 U/ml 0.05 M $NaHCO_3$) neither influenced the secretory volume nor protein or lipase output (Fig. 2).

Effect of intraduodenal infusion of trypsin and trypsin inhibitor on pancreatic enzyme secretion

Intestinal infusion (1.2 ml/h) of trypsin (350 U/ml 0.05 $NaHCO_3$) in a dose calculated to correspond to twice the amount of trypsin(ogen) spontaneously secreted significantly suppressed secretory volume, protein output, protein concentration, and trypsinogen output (Fig. 3 A-D). The infusion of trypsin inhibitor in the presence of intraduodenal trypsin abolished the inhibitory effect exerted by trypsin.

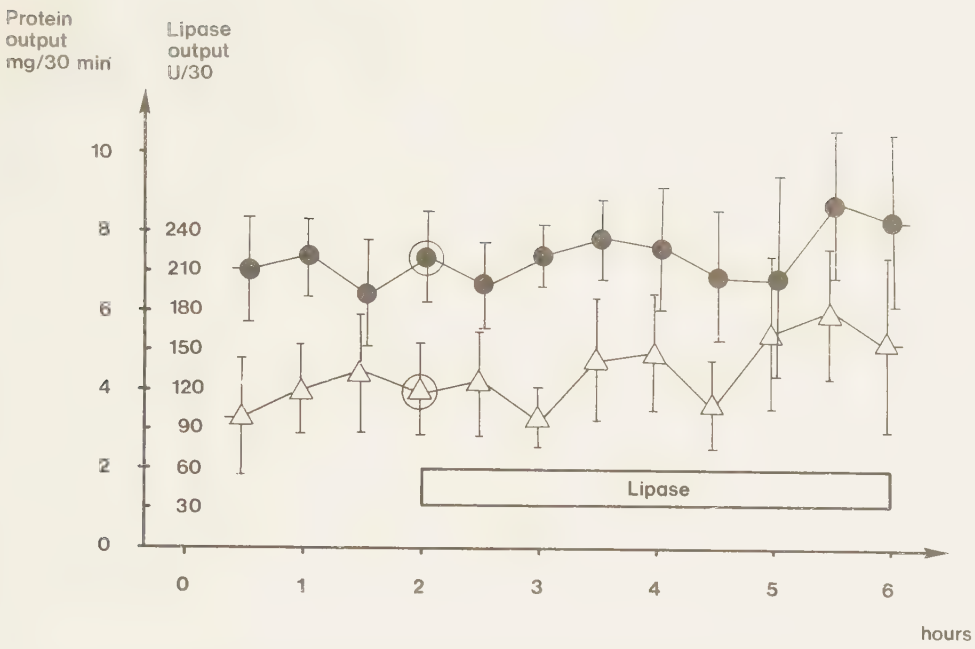


Fig. 2 Influence on protein (●—●) and lipase (△—△) output of duodenal infusion of lipase in rats provided with bile-pancreatic fistulae (n = 5). Values are given as mean ± SEM. The circles indicate the preinfusion values.

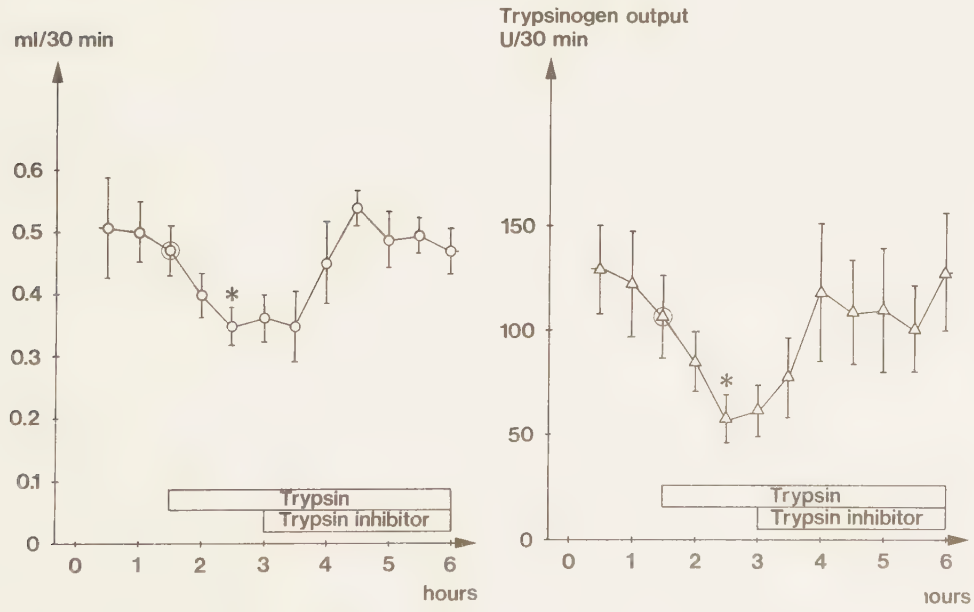


Fig. 3 A - B Influence on protein output and protein concentration of intraduodenal infusion of trypsin and trypsin inhibitor in rats provided with bile-pancreatic fistulae (n = 5). Values are given as mean ± SEM. The circles indicate the preinfusion values used in the statistical calculations.
* = $p<0.05$ ** = $p<0.01$

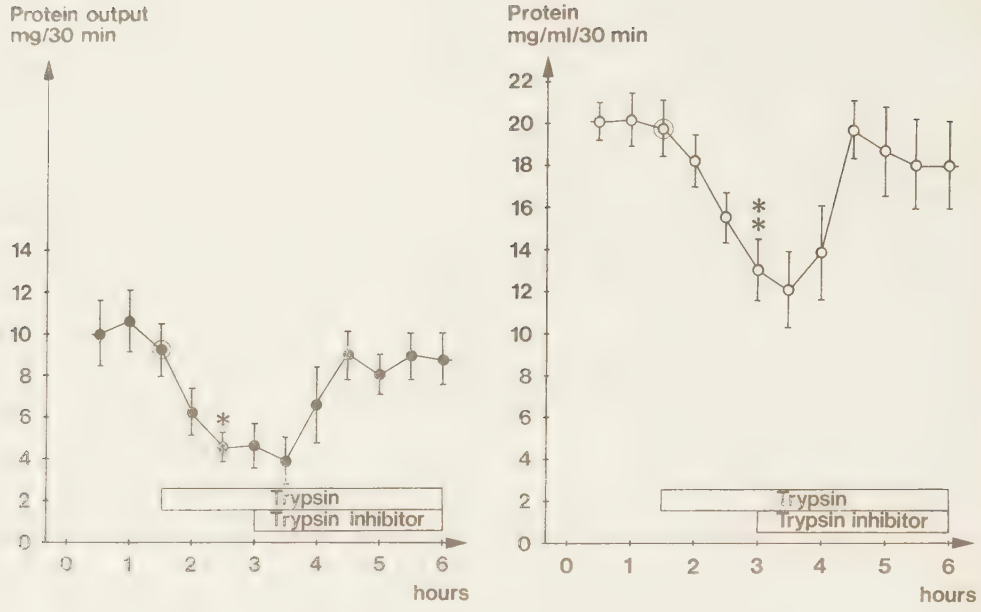


Fig. 3 C - D Influence on secretory volume and trypsinogen output of intraduodenal infusion of trypsin and trypsin inhibitor in rats provided with bile-pancreatic fistulae (n = 5). Values are given as mean ± SEM. The circles indicate the preinfusion values used in the statistical calculations.
* = $0 < 0.05$

Effect of intraduodenal infusion of bile on pancreatic enzyme secretion in the abscence or presence of intraduodenal trypsin

Duodenal infusion of pure rat-bile (1.2 ml/h) free from pancreatic enzymes did neither affect the secretory volume (data not shown) nor the protein or amylase output (Fig. 4). As pancreatic secretion probably is maximally stimulated in rats with diverted bile-pancreatic juice (21) the same experiment was performed during trypsin infusion (2.5 mg/ml 0.05 M $NaHCO_3$) into the duodenum. Not even in this experiment bile infusion influenced the secretion (Fig. 5)

DISCUSSION

This study confirms that trypsin within the intestine markedly inhibits pancreatic enzyme secretion also in a dose (2.5 mg/ml) significantly lower than that (10 mg/ml) used in previous experiments (15). New findings from this study are that intraduodenal infusion of amylase or lipase - in doses equivalent to those of trypsin - in pancreatic fistula rats seems not to influence the secretory volume or enzyme output. Also during inhibition of pancreatic enzyme secretion by intraluminal trypsin introduction of bile into the duodenum did not affect the secretion.

In recent years it has come to be recognized that intraluminal trypsin exerts a feed back regulation on pancreatic secretion in rat (11, 15),

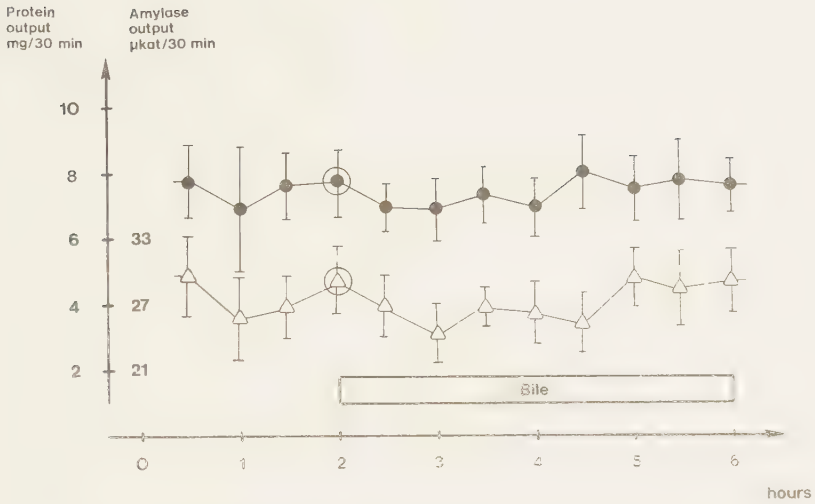


Fig. 4 Influence of duodenal infusion of bile on protein and amylase output in rats provided with bile-pancreatic fistulae (n = 5). Values are given as mean ± SEM. The circles indicate the preinfusion values. ●—● = protein output △—△ = amylase output

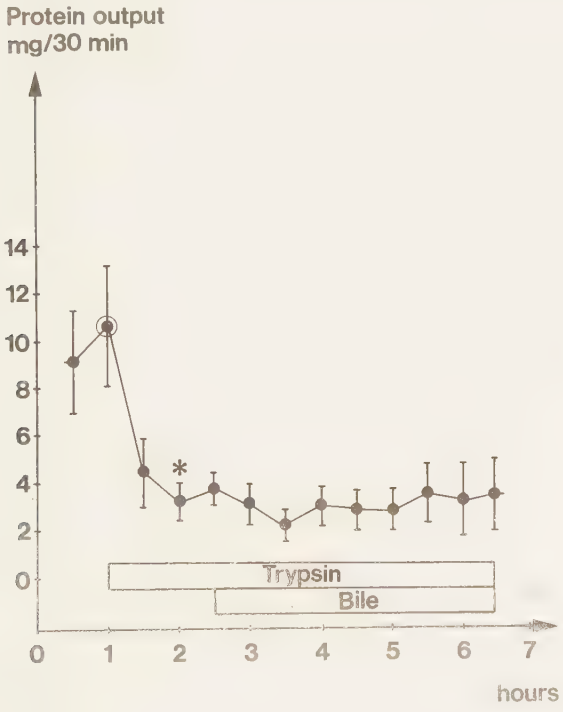


Fig. 5 Effect on protein output of intraduodenal infusion of trypsin and bile in rats provided with bile-pancreatic fistulae (n = 5). Values are given as mean ± SEM. The circles indicate the preinfusion values used in the statistical calculations.
* = $p<0.05$

pig (13) and probably man (14). Hitherto it has not been clear if other components of the pancreatic juice than proteolytic enzymes influence pancreatic secretion in the rat. In the pig infusion of amylase into the duodenum did not change pancreatic secretory volume or protein output (13) which is in accordance with the finding of the present study. In long-term experiments, however, Macri et al. (17) observed pancreatic hypertrophy and increased pancreatic amylase content in chicken fed albumin amylase inhibitor. Whether these discrepancies are due to species differences or reflect contamination of the amylase inhibitor preparation with trypsin inhibitors in the latter experiments cannot be judged at the moment.

There are no reports in the literature concerning the relation of intraluminal lipase to pancreatic secretion. The lipase used in the present study was of pancreatic origin and free of co-lipase activity and other pancreatic enzymes. It is well established that purified pancreatic lipase is inhibited by conjugated bile salts in concentrations above their critical miscellar concentration (2). However, as bile was deviated throughout the present experiments the lipolytic activity administered ougth to be unchanged when reaching the intestinal lumen.

Staub and Sarles (22) studied the influence of bile on the pancreatic secretion in the rat. Their observation that the presence of bile in the duodenum inhibits the pancreatic secretion of protein is inconsistent with the findings of the present study. On the other hand, infusion of intraduodenal bile or bile salts has been shown to stimulate the pancreatic secretion in cat (18) and man (10, 20). In our previous studies (15) we, like Green and Lyman (11) and Petersen and Grossman (21), have found that recirculation of bile-pancreatic juice markedly inhibits the pancreatic enzyme secretion in the rat. These observations are in conformity with the results of the present study that trypsin also in combination with bile inhibits pancreatic enzyme secretion. In man Osnes (19) found that a stimulatory effect of bile on the pancreatic secretion was reversed following intraduodenal infusion of pancreatic juice.

In summary, it was found in conscious rats surgically prepared so that substances could be infused into the duodenum and the secretion could be collected through a bile-pancreatic duct fustula, that intraduodenal infusion of trypsin in contrast to equivalent doses of amylase and lipase suppressed pancreatic enzyme secretion markedly. Furthermore it was found that the infusion of bile into the duodenum did not affect the secretion either in the abscence or presence of intraluminal bile.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Medical Research Council (Project No. 17X-03012), the Medical Faculty of Lund, the Albert Påhlsson Foundation and the Harald and Greta Jeansson Foundation.

REFERENCES

1. Arnesjö B, Ihse I, Lundquist I & Quist I. Scand J Gastroent 1973, 8, 545-554

2. Borgström B & Erlanson C. Biochim Biophys Acta 1971, 242, 509-604

3. Ceska M, Birath K & Brown B. Clin Chim Acta 1969, 26, 437-444

4. Chernick S S, Lepkovsky S & Chaikoff I L. Amer J Physiol 1948, 155, 33-41

5. O'Donnel M D, Fitzgerald O & McGeeney . Scand J Gastroent 1977, 12, 683-687

6. Donnér J. Acta Chem Scand 1976, B30, 430-434

7. Eggstein M & Creutz F M. Klin Wchr 1955, 33, 879-884

8. Erlanson C & Borgström B. Scand J Gastroent 1970, 5, 293-295

9. Frey E K, Kraut H & Werle E. Das Kallikrein-Kinin-System und seine Inhibitoren, pp 126. Ferdinand Enke Verlag, Stuttgart, 1967

10. Forell M M, Otte M, Kohl H J, Lehnert P & Stahlheber H P. Scand J Gastroent 1971, 6, 261-265

11. Green G M & Lyman R L. Proc Soc Exp Biol Med 1972, 140, 6-12

12. Hummel B W C. Canad J Biochem Physiol 1959, 37, 1393-1399

13. Ihse I & Lilja P. Scand J Gastroent 1979, in press

14. Ihse I, Lilja P & Lundquist I. Digestion 1977, 15, 303-308

15. Ihse I, Lilja P & Lundquist I. Scand J Gastroent 1979, in press

16. Lowry O H, Rosenbrough N J, Farr A C & Randall R J. J Biol Chem 1951, 193, 365-375

17. Macri A, Parlamenti R, Silano V & Valfre F. Poultry Science 1977, 56, 434-441

18. Mellanby J. J Physiol 1926, 61, 419-435

19. Osnes M. VI World Congress of Gastroenterology, Abstract p 6

20. Osnes M, Hansen L E, Flaten O & Myren J. Gut 1978, 19, 180-184

21. Peterson H & Grossman M I. Am J Physiol 1977, 233 (6), E 530-E 536

22. Staub J L & Sarles H. European Pancreatic Club, 1978, Abstract p 48

23. Wilson P A, Melmed R N, Hampe M M W & Holt S J. Gut 1978, 19, 260-266

TRYPSIN AS A REGULATOR OF PANCREATIC ENZYME SECRETION IN THE RAT

Ingemar Ihse, Per Lilja and Ingmar Lundquist

Departments of Surgery and Pharmacology,
University of Lund, S-221 85 Lund, Sweden

Abstract

The effect of intraduodenally administered trypsin on pancreatic enzyme secretion was investigated in conscious rats surgically prepared with bile-pancreatic fistulae.

Introduction of $NaHCO_3$ into the duodenum did not influence the secretion Reintroduction of bile-pancreatic juice into the duodenum, however, suppressed the protein output mainly due to changes in protein concentration. Infusion of trypsin into the duodenum in the abscence of intraluminal pancreatic juice significantly suppressed the secretory volume and pancreatic enzyme output; addition of trypsin inhibitor to the trypsin infusate resulted in an immediate increase of the secretion. Trypsin inhibitor per se, however, was without effect.

Bile-pancreatic juice affected amylase, lipase and trypsinogen output in a parallel fashion; after addition of trypsin inhibitor to the infusate the inhibitory effects on pancreatic enzyme output was reversed in a parallel manner. The results support the hypothesis that pancreatic exocrine secretion is regulated via a feed-back mechanism exerted - at least partly - by intraluminal trypsin.

Key-words: Amylase; Feed-back regulation; Intraluminal trypsin; Lipase; Pancreatic protein output; Trypsinogen.

Reprint requests: Per Lilja, M.D., Department of Surgery, University of Lund, S-221 85 Lund, Seden

It is well established that feeding of trypsin inhibitor to chickens and rats results in pancreatic hypertrophy (3,15,23) and increased capacity of the pancreas to secrete digestive enzymes (27). Based on studies in rats a negative feed-back regulation of pancreatic enzyme secretion exerted through variations in the intraluminal trypsin concentrations has been suggested as an explanation of these findings (11). Recently we have presented evidence of a similar mechanism operating in pig (18) and man (19).

The purpose of the present study was to further elucidate in conscious rats the interrelationships between the intraduodenal pancreatic enzyme milieu and pancreatic secretion with special reference to the effects of trypsin, bovine lung trypsin inhibitor, and bile-pancreatic juice. In some experiments the pancreatic output of both amylase, lipase and trypsinogen activities was recorded to study whether the present experimental conditions would favour parallel or non-parallel enzyme secretion.

MATERIAL AND METHODS

Animals

Male Wistar rats, weighing between 250 and 300 grams were purchased from a local supplier. Food and water was withdrawn from the animals immediately before the surgical procedure. The animals were anesthetized with ether and an abdominal wall incision was made exposing the duodenum and the bile-pancreatic duct. A poly-ethylene catheter with an inner diameter of 0.58 mm and a length of 15 cm (Intramedic PE-50) was introduced into the bile-pancreatic duct via an incision close to the intestinal wall. Another catheter was introduced into the duodenum 5 mm below pylorus to permit direct infusion into the intestine. Separate skin incisions were used for the passage of the cannulae. The animals were maintained in restraining cages and allowed at least 12 hours of surgical recovery. Solid food was withdrawn from the animals but they had free access to fluid composed of one part Ringer solution and one part 5.5% glucose solution.

Intestinal infusates

Porcine crystalline trypsin obtained from A/S Novo, Copenhagen, Denmark, was dissolved in 0.05 M $NaHCO_3$ to a concentration of 10 mg/ml. The tryptic activity of this solution was 1400 U/ml as measured with the method described below (14). Highly purified bovine lung trypsin inhibitor was obtained from Bayer AG, Leverkusen, Germany. The inhibitor is not absorbed from the intestine (32) or digested by intestinal proteolytic enzymes and inhibits both trypsin and chymotrypsin at a pH optimum of 8.0 (8). In our laboratory ancillary experiments showed that 0.5 mg of the inhibitor completely abolished the tryptic activity of 1 mg of trypsin. Bile-pancreatic juice used for intraduodenal infusion was collected on ice the day before the experiments and frozen at -20°C. The pH of all infusates used was 8.0±0.2 and the temperature was brought to 25°C immediately before the infusion into the duodenum.

Experimental procedure

All experiments were performed 12 hours after the surgical procedure and only one experiment was performed in each animal. Animals in which complications occurred such as catheter dislodgement or bleeding were excluded from the experiments. The infusions were carried out using an infusion pump with a constant rate of 1.2 ml/h which roughly corresponded to the spontaneous volume secreted via the bile-pancreatic duct fistula. Bile-pancreatic secretion was collected in small tubes on ice at 30 minutes intervals. The samples were immediately frozen at -20°C and analyzed in duplicate within 2 weeks.

Laboratory techniques

The volume of the bile-pancreatic juice secreted during the experimental period was measured every 30 minutes. Protein was assayed according to the method described by Lowry et al (22), as modified by Eggstein and Creutz (6). Lipase was assayed by a scaled-down version of the method described by Erlanson and Borgström using tributyrine as the substrate (7). Amylase was determined with the Phadebas® test (2), a gift from AB Pharmacia, Uppsala, Sweden. Trypsinogen was estimated by activation of zymogens in bile-pancreatic juice by enterokinase (Miles Laboratories, USA). A mixture of 100 µl diluted bile-pancreatic juice (1:10), 200 µl 0.05 M Tris-HCl buffer, pH 8.0, containing 0.02 M $CaCl_2$ and 200 µl enterokinase (0.05 mg/ml in redistilled water) was incubated at 25°C for 2 hours. The reaction was stopped by addition of 10 µl 2 M HCl. The tryptic activity was determined by a modified Hummel method using TAME (p-toluene-sulphonyl-L-arginine methyl ester) as the substrate (14).

Calculations

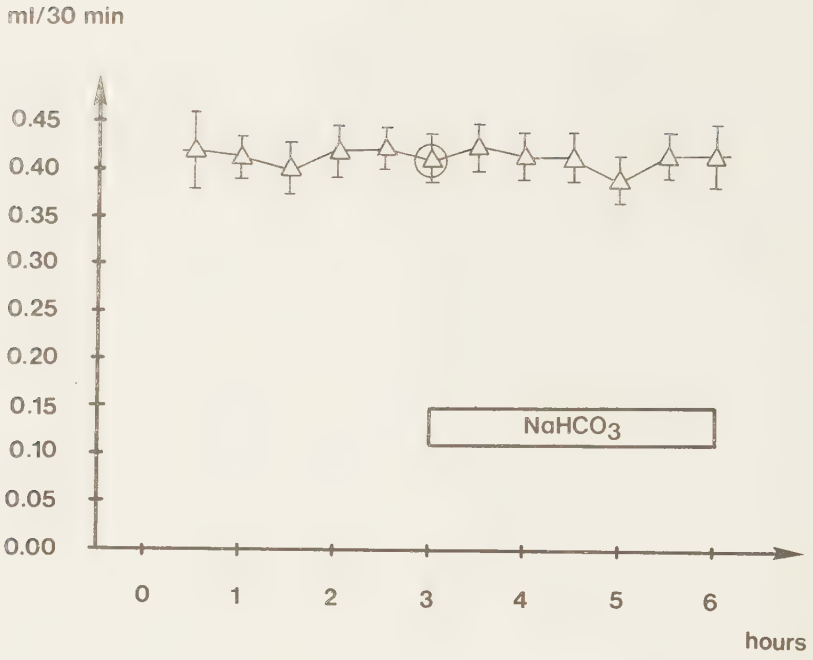
The data obtained were subjected to statistical analysis using the Student's t-test. All values are given as mean ± S.E.M.

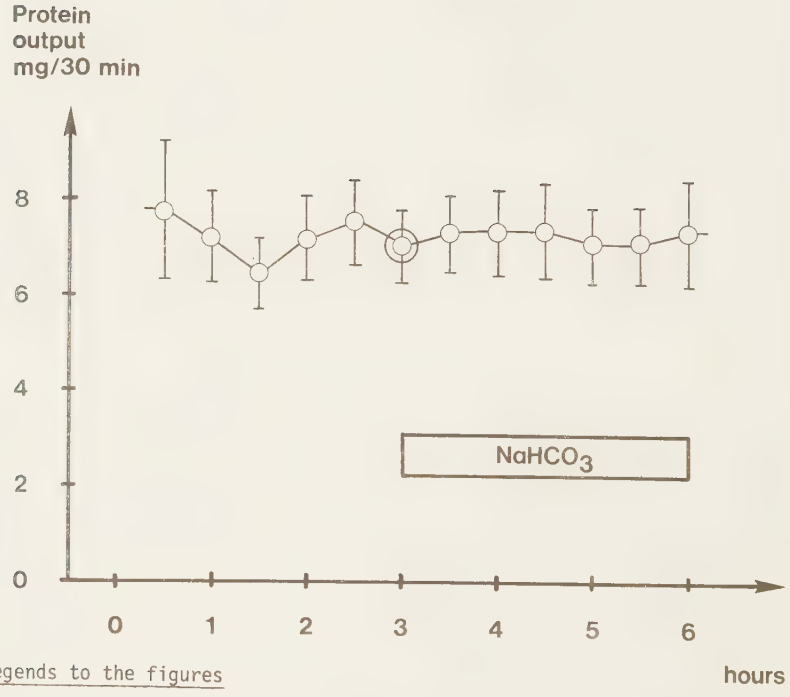
RESULTS

Deviation of bile-pancreatic juice from the intestine; intraduodenal infusion of sodium-bicarbonate

An initial increasing secretion of protein followed the exclusion of bile-pancreatic juice from the intestine. However, after about 10 hours a stable level of hypersecretion was reached lasting for at least 48 hours. All experiments were performed 12 hours after diversion of the bile-pancreatic secretions. The stable secretory pattern during the experimental period is shown in Fig. 1.

Intraduodenal infusion of 0.05 M $NaHCO_3$ (Fig. 1) did not result in any alteration of volume, protein concentration, or protein output of the bile-pancreatic secretion.





Legends to the figures

Figure 1. Secretory volume and protein output in 7 rats with bile-pancreatic fistulae. Sampling started 12 hours after surgery. 1.2 ml/hour of 0.05 M $NaHCO_3$ pH 8.0 ± 0.2 was infused intraduodenally. The circles represent the preinfusion points. Values are given as mean ± SEM.

Reintroduction of bile-pancreatic juice into the duodenum

The next series of experiments was designed to study the secretion after reintroduction of bile-pancreatic juice into the duodenum. A highly significant suppression of protein output occurred after one hour of reintroduction and remained throughout the experiment (Fig. 2). There was only a slight effect on the secretory volume but the protein concentration of the bile-pancreatic secretion decreased significantly.

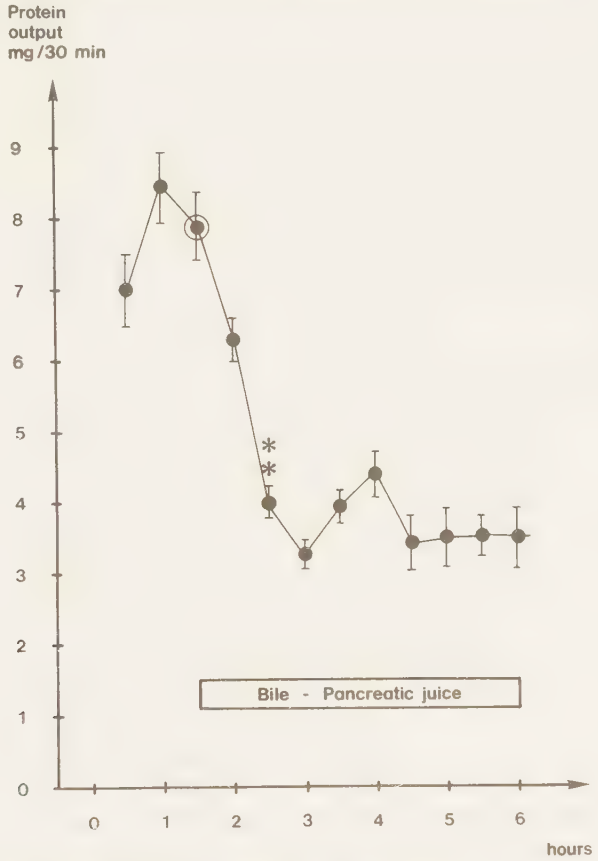


Figure 2.

Pancreatic protein output in 11 rats with bile-pancreatic juice deviated. 1.2 ml/hour of bile-pancreatic juice was reintroduced into the duodenum. The circle represents the preinfusion point. Values are given as mean ± SEM.
** = $p < 0.01$

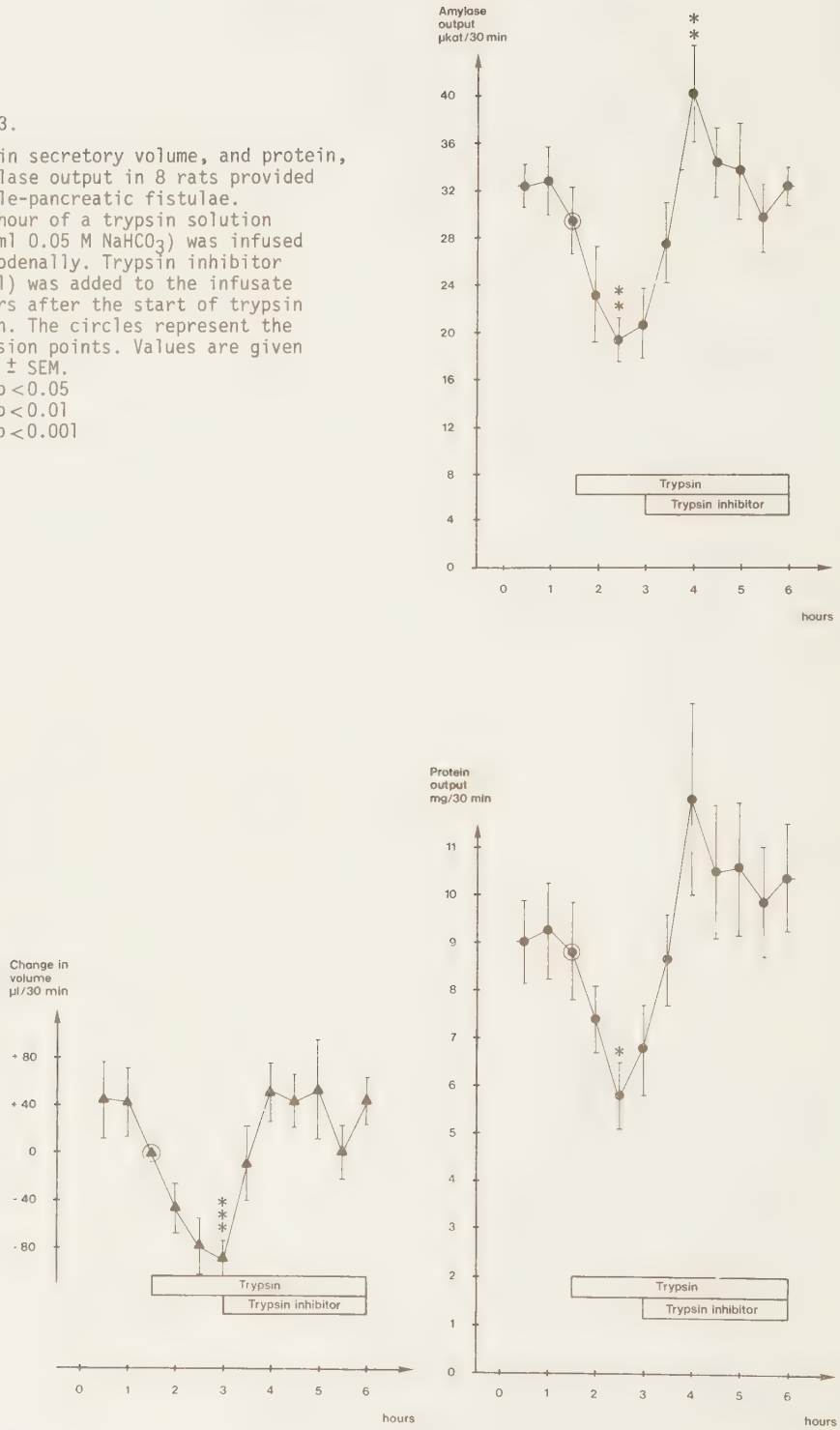
Infusion of trypsin and trypsin inhibitor into the duodenum

The effect on pancreatic secretion of trypsin infusion into duodenum in the absence of intraluminal bile-pancreatic juice was then investigated. It was observed that infusion of trypsin alone effectively suppressed both the secretory volume and the enzyme secretion expressed as protein and amylase output (Fig. 3). Addition of trypsin inhibitor to the infusate resulted in an immediate increase of pancreatic secretion to a level comparable to that found prior to the trypsin infusion. An even more pronounced secretion was observed one hour after starting the inhibitor infusion (Fig. 3). To elucidate if trypsin inhibitor per se had a secretagogue effect bovine lung trypsin inhibitor was infused into the duodenum. No effect on the pancreatic secretion was noted (data not shown).

Figure 3.

Change in secretory volume, and protein, and amylase output in 8 rats provided with bile-pancreatic fistulae. 1.2 ml/hour of a trypsin solution (10 mg/ml 0.05 M $NaHCO_3$) was infused intraduodenally. Trypsin inhibitor (5 mg/ml) was added to the infusate 1.5 hours after the start of trypsin infusion. The circles represent the preinfusion points. Values are given as mean ± SEM.

* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$



Reintroduction of bile-pancreatic juice and trypsin inhibitor into the duodenum

The last series of experiments was designed to study the effects on pancreatic secretion of trypsin inhibitor infusion after reintroduction of bile-pancreatic juice into the duodenum (Fig. 4). A highly significant reduction of the protein output and protein concentration occurred.

The effect of returning bile-pancreatic juice on the output of individual pancreatic enzymes i.e. amylase, lipase and trypsinogen was also recorded. A pronounced and apparently parallel inhibition of the output of all 3 enzymes was observed within one hour (Fig. 4 and table I). After addition of trypsin inhibitor to the infusate the inhibitory effects on the output of protein and the different enzymes studied was reversed in a parallel manner reaching levels comparable to those measured before reinfusion of bile-pancreatic juice (Table I).

DISCUSSION

The results of the present paper show that intraduodenal infusion of $NaHCO_3$ in rats provided with bile-pancreatic fistulae did not influence bile-pancreatic secretion with regard to secretory volume, protein concentration or protein output. Reintroduction of bile-pancreatic juice into the duodenum caused a marked inhibition of the protein output.

Trypsin infusion into the duodenum markedly depressed the secretory volume and protein and amylase concentrations in the pancreatic juice. Addition of trypsin inhibitor to either trypsin infusion or infusion of bile-pancreatic juice caused a dramatic increase in the protein and enzyme secretion of the pancreas. Bovine trypsin inhibitor alone induced no secretagogue effects on the pancreas. An apparently parallel inhibition and stimulation, respectively, of amylase, lipase and trypsinogen secretion was observed.

It is known that feeding of trypsin inhibitor to rats and chickens results in morphological and functional pancreatic changes such as hypertrophy (3), increased enzyme concentrations and increased secretory capacity of the pancreas (23). In 1958 Grossman (12) observed pancreatic hypersecretion in rat with chronic pancreatic fistulae. Later Green and Lyman (11) proposed that pancreatic enzyme secretion in the rat was controlled by a feed-back mechanism. Their results are in accordance with the findings of the present study that trypsin - at least partly - might play a regulatory role. Our experiments, however, revealed that the secretory volume was suppressed following infusion of trypsin. This is in contrast to results reported by Green and Lyman (11) who found no changes in the secretory volume after intraduodenal trypsin infusion in the rat. If only proteolytic enzymes are involved in this regulatory mechanism or if other enzymes or factors also are of importance remains to be elucidated (21).

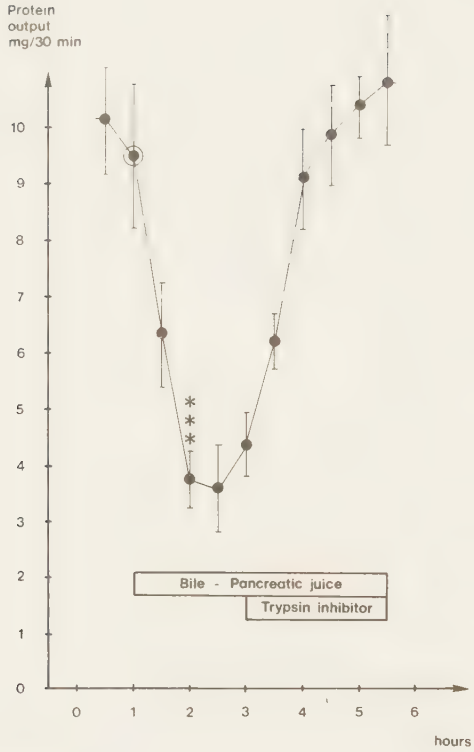
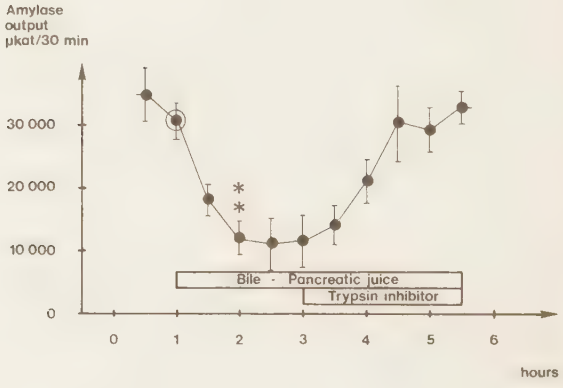


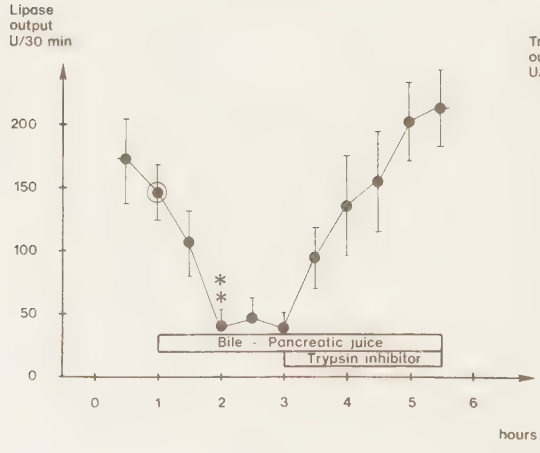
Figure 4.

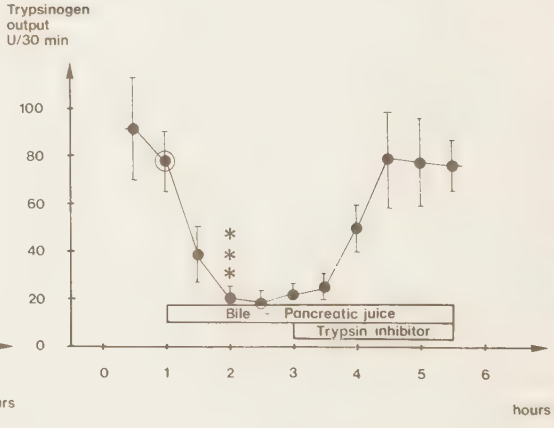
Pancreatic protein, amylase, lipase, and trypsinogen output in 5 rats provided with bile-pancreatic fistulae. 1.2 ml/hour of bile-pancreatic juice was reintroduced intraduodenally. Trypsin inhibitor was added to the infusate 2 hours after start of the reintroduction. The circles represent the preinfusion points. Values are given as mean ± SEM.

** = $p < 0.01$

*** = $p < 0.001$







	Pre-infusion level	Bile - pancreatic juice				Bile - pancreatic juice + + trypsin inhibitor		
Hours	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Amylase output	100±11	59±9	38±6	35±11	37±11	45±7	67±7	98±20
Lipase output	100±13	73±17	27±8	20±9	26±5	66±15	93±22	106±31
Trypsinogen output	100±10	49±10	26±7	23±8	30±5	34±8	77±12	103±25

Table I. The change in pancreatic amylase, lipase, and trypsinogen output expressed as per cent in 5 rats provided with bile-pancreatic fistulae. Bile-pancreatic juice was reintroduced into the duodenum without and with the addition of trypsin inhibitor. No statistical differences were found at any time interval between the values of the different enzymes.

Most studies on the regulation of pancreatic secretion have been performed in dogs. Annis and Hallenbeck (1) and Hong et al (13) reported that in dogs with pancreatic fistulae introduction of pancreatic juice into the duodenum inhibited pancreatic secretion. On the other hand Sale et al (28) in acute and chronic experiments in dogs found no evidence of exocrine pancreatic feed-back inhibition. Magee and Hong, however, in pigs with pancreatic fistulae found a marked depression of pancreatic secretory volume and amylase secretion following intraduodenal reinfusion of pancreatic juice (24). Corring reported similar observations in the pig (4). In a recent study in the pig we found that also in this species pancreatic secretion is subject to a feed-back inhibition from intestinal trypsin (18). Studies on the recovery of pancreatic enzymes when infused into the duodenum of human subjects with ileostomy yielded results suggestive of a pancreatic feed-back inhibition in man (9). Recently the results from a study of a patient in our laboratory gave more direct evidence that the proposed pancreatic feed-back mechanism also exists in man (19).

The concept of a parallel secretion of the different pancreatic enzymes has been accepted for a long time. Such an opinion is in conformity with the theory of exocytosis (20). Recently, however, a non-parallel enzyme secretion has been reported by several authors in different investigations (5,10,26). In previous experiments we found that long-term oral trypsin inhibitor administration to rats induced a non-parallel increase of enzyme concentrations within the pancreatic tissue. Thus trypsinogen increased in average 150% while lipase and amylase increased in average 30% (16). In the present series of acute experiments we found a parallel

secretion of trypsinogen, amylase and lipase both when the pancreatic secretion was inhibited by intraluminal reintroduction of bile-pancreatic juice and when it was stimulated by the addition of trypsin inhibitors to the infusates. Thus the adaptation of the pancreatic enzyme content to chronic stimuli by trypsin inhibitors was not reflected in similar changes of the secretory enzyme pattern in acute experiments.

In our experiments a stable state of hypersecretion was reached about 10 hours after deviation of bile-pancreatic juice. This experimental design with a stable secretory level seems to be a valid model to study changes in the pancreatic secretion since in addition to the inhibitory effects studied in the present investigation it has been shown that exogenous CCK-PZ evoked a strong enzyme output in animals with their pancreatic secretion diverted for more than 10 hours (29).

The mechanism by which trypsin suppresses pancreatic enzyme secretion is not fully understood. It has been suggested that trypsin inhibitor induced pancreatic secretion is mediated by release of one or more intestinal factors or hormones (23). In previous studies we showed that long-term parenteral trypsin inhibitor administration in the rat did not induce pancreatic hypertrophy in contrast to oral administration (16) and that resection of the proximal third of the small intestine abolished the effects of the pancreas induced by oral trypsin inhibitor administration (15). As most gastrointestinal hormones are localized to the proximal third of the small intestine (30) our previous experiments speak in favour of a hormonal mediation of the pancreatic feed-back regulation. Recently it has been shown in rats and dogs that long-term cholecystokinin and caerulein administration induced morphological and functional changes in the pancreas resembling those seen in the rat after trypsin inhibitor administration (17,25,31). Wilson et al (32) in immunocytochemical studies in rats found that trypsin inhibitors did not enter the mucosal cells. These results are consistent with an intraluminal mode of action of the trypsin inhibitor. Thus it seems logical to assume that the pancreatic response to intraluminal trypsin inhibitor primarily is a result of inhibition of tryptic activity.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Medical Research Council (Projects No 17X-03012 and 04P-4289), the Medical Faculty of Lund and the Albert Påhlsson Foundation.

REFERENCES

1. Annis, D. and Hallenbeck, G.A.:
Proc. Soc. Exp. Biol. Med. 1951, 77, 383-385

2. Ceska, M.; Birath, K. and Brown, B.:
Clin. Chim. Acta 1969, 26, 437-444

3. Chernik, S.S.; Lepkovsky, S. and Chaikoff, I.L.:
Am. J. Physiol. 1948, 155, 33-41

4. Corring, T.:
Ann. Biol. Anim. Biochim. Biophys. 1973, 13, 755-756

5. Dagorn, J.C.; Langier, R.; Demol, P.; Figarella, C. and Sarles, H.:
Digestion 1978, 17, 451-452

6. Eggstein, M. and Creutz, F.M.:
Klin. Wschr. 1955, 33, 879-884

7. Erlanson, C. and Borgström, B.:
Scand. J. Gastroent. 1970, 5, 293-295

8. Frey, E.K.; Kraut, H. and Werle, E.:
Das Kallikrein-Kinin-System and seine Inhibitoren.
Ferdinand Enke Verlag, Stuttgart, 1967

9. Goldberg, D.M.; Campbell, R. and Roy, A.D.:
Gut 1969, 10, 477-483

10. Goldberg, D.M. and Wormsley, K.G.:
Gut 1970, 11, 859-866

11. Green, G.M. and Lyman, R.L.:
Proc. Soc. Exp. Biol. Med. 1972, 140, 6-12

12. Grossman, M.I.:
Am. J. Physiol. 1958, 194, 535-539

13. Hong, S.S.; Nakamura, M. and Magee, D.F.:
Ann. of Surg. 1967, 166, 778-782

14. Hummel, B.W.C.:
Canad. J. Biochem. Biophys. 1959, 37, 1393-1399

15. Ihse, I.:
Scand. J. Gastroent. 1976, 11, 11-17

16. Ihse, I.; Arnesjö, B. and Lundquist, I.:
Scand. J. Gastroent. 1974, 9, 719-724

17. Ihse, I.; Arnesjö, B. and Lundquist, I.:
Scand. J. Gastroent. 1976, 11, 529-535

18. Ihse, I. and Lilja, P.:
To be published

19. Ihse, I.; Lilja, P. and Lundquist, I.:
Digestion 1977, 15, 303-308

20. Jamieson, J.O. and Palade, G.E.:
J. Cell Biol. 1971, 48, 503-522

21. Lilja, P.:
To be published

22. Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L. and Randall, R.J.:
J. Biol. Chem. 1951, 193, 265-271

23. Lyman, R.L. and Lepkovsky, S.:
J. Nutr. 1957, 62, 269-284

24. Magee, D.F. and Hong, S.S.:
Swine in Biomedical Research 1965, 109-119

25. Ribet, A.; Fedou, R. and Frexinos, J.:
Digestion 1969, 2, 145-158

26. Rinderknecht, H.; Renner, J.G.; Douglas, A.P. and Adham, N.F.:
Gastroenterology 1978, 75, 1083-1089

27. Rothman, S.S. and Wells, H.:
Am. J. Physiol. 1969, 216, 504-507

28. Sale, J.K.; Goldberg, D.M.; Fawcett, A.N. and Wormsley, K.G.:
Digestion 1977, 15, 540-555

29. Schneeman, B.O. and Lyman, R.L.:
Proc. Soc. Exp. Biol. Med. 1975, 148, 897-903

30. Schneeman, B.O. and Lyman, R.L.:
Digestion 1977, 15, 28-33

31. Tardini, A.; Anversa, P.; Bordi, D.; Bertaccini, G. and Impicciatore, M.:
Am. J. Path. 1971, 62, 35-48

32. Wilson, P.A.; Melmed, R.N.; Hampe, M.M.V. and Holt, S.J.:
Gut 1978, 19, 260-266

Accepted for publication in Scandinavian Journal of Gastroenterology.

VAGAL INFLUENCES ON THE PANCREATIC RESPONSE TO INTRALUMINAL TRYPSIN

I. Ihse, P. Lilja and I. Lundquist

Departments of Surgery and Pharmacology,
University of Lund, Lund, Sweden

Abstract

The significance of vagal influences on the pancreatic response to intraluminal trypsin was investigated in conscious rats surgically prepared with bile-pancreatic fistulae. Vagotomy as well as cholinergic blockage depressed the hypersecretion of protein in fistula rats. Contrary to in control rats intraduodenal trypsin infusion did not change the protein output after vagotomy or cholinergic blockage. Indirect vagal stimulation induced by insulin hypoglycaemia did not futher increase the protein output in rats with their bile-pancreatic juice deviated from the intestine. In these latter rats, however, intraduodenal infusion of trypsin markedly inhibited protein secretion. The results suggest that vagal integrity is essential for the response of the pancreas to intraduodenal trypsin.

Key-words: Cholinergic blockage; Cholinergic stimulation; Feed-back; Intraduodenal; Pancreatic secretion; Trypsin; Vagotomy.

Reprint requests: Per Lilja, M.D., Department of Surgery, University of Lund, S-221 85 Lund, Sweden

Long-term oral administration of various trypsin inhibitors causes pancreatic hypertrophy in rat and chicken (1, 4, 24). Green and Lyman (8) suggested a feed-back regulation of pancreatic enzyme secretion exerted by intraduodenal trypsin as a mechanism for this trypsin inhibitor-induced effect. Recently similar effects of trypsin have been demonstrated in pig (16) and man (17). The mechanisms by which trypsin exerts these changes are only partially elucidated (18, 21). Data hitherto published suggest a hormonal mediation. Whether in addition neural factors influence the pancreatic response to intraluminal trypsin is, however, unknown. In the present study the effect of vagotomy, cholinergic blockage and insulin hypoglycaemia on the observed feed-back regulation was investigated.

MATERIAL AND METHODS

Surgical procedures

Male Wistar rats weighing between 250 and 300 g were used and one experiment was performed on each rat. Clean but not sterile instruments were used. Food and water was withdrawn from the animals immediately before the surgical procedures. Ether anaesthesia was used and an abdominal incision was made, exposing the bile-pancreatic duct and the duodenum. A polyethylene catheter with an inner diameter of 0.58 mm and a length of 15 cm (Intramedic PE 50) was introduced into the bile-pancreatic duct where it enters the intestine. Another catheter was inserted into the duodenum 5 mm below pylorus to permit direct duodenal administration.

In some rats both vagal truncs were sectioned just below the diaphragm according to the method described by Shay et al. (28). The animals were maintained in restraining cages and allowed 14 to 15 hours of postoperative recovery. No antibiotics were used. As vagotomized rats have impaired emptying of the stomach 15 ml of saline was injected subcutaneously. Food but not fluid (1 part of Ringer's solution and 1 part of 5.5 % glucose solution) was withdrawn from the animals during the recovery period while they were fasted during the experiments.

Drugs and chemicals

The anticholinergic drug propantheline (2-hydroxy-ethyl) diisopropyl-methyl-ammonium bromide xanthene-9-carboxylate (Pro-Banthine®, G.D. Searle & Co, Chicago, Ill., USA) was used. Insulin Vitrum® was obtained from AB Vitrum, Stockholm, Sweden. Porcine crystalline trypsin (A/S Novo, Copenhagen, Denmark) was dissolved in 0.05 M $NaHCO_3$ at a concentration of 5 mg/ml corresponding to a tryptic activity of 700 U/ml as analyzed with TAME (p-toluene-sulphonyl-L-arginine methyl ester) as the substrate (12). Highly purified bovine lung trypsin inhibitor was obtained from Bayer AG, Leverkusen, Germany. The inhibitor is not absorbed from the intestine or digested by intestinal proteolytic enzymes (7). In ancillary experiments it was found that 0.5 mg of the inhibitor completely abolished the tryptic activity of 1 mg of the trypsin used.

Experimental design

In the first experiment the effect of cholinergic blockage on bile-pancreatic secretion was studied in 10 fistula rats. The bile-pancreatic secretion was collected in 30 min intervals. After 60 min Pro-Banthine® was given subcutaneously in a dose of 4 mg/kg and the secretion collected for another 120 min.

In the second series of experiments the effect of intraduodenal trypsin infusion on bile-pancreatic secretion was studied after cholinergic blockage, after vagotomy or during insulin-induced hypoglycaemia. In all experiments the pH of the infusates was corrected to 8.0 ± 0.2 and the temperature was brought to +25°C immediately before introduction into the duodenum. The infusions were carried out at a constant rate of 1.2 ml/h which roughly corresponded to the volume spontaneously secreted from the bile-pancreatic fistula. Eight rats served as control rats. Nine rats were subjected to vagotomy. In all animals used the vagotomy was found to be complete according to the method described by Håkanson et al. (10). Eight rats were given Pro-Banthine® (4 mg/kg) subcutaneously 30 minutes before the start of the experiment. Hypoglycaemia was induced in 8 rats by repeated subcutaneous injections of 1 U of insulin every second hour. The first injection was given 30 minutes before the start of the experiment. Also these rats were otherwise treated exactly as the control group.

Evaluation of pancreatic secretion

In all experiments the bile-pancreatic secretion was collected in small pre-weighed tubes on ice in 30 min intervals. The samples were immediately frozen at -20°C and analyzed in duplicate within 2 weeks. The volume of the bile-pancreatic juice was estimated by weight and the concentration of protein determined according to the method described by Lowry et al. (22) as modified by Eggstein and Creutz (6).

In the present series of experiments the combined bile-pancreatic secretion was collected to avoid problems with the low flow obtained when pure pancreatic juice is collected in the rat. In previous experiments in rats provided with bile-pancreatic fistulae protein output was found to be secreted essentially in parallel with pancreatic enzymes (amylase, lipase and/or trypsinogen) (18). In this study only protein output was determined and considered to roughly reflect pancreatic enzyme secretion.

Calculations

The data obtained were subjected to statistical analysis using the Student's *t*-test for paired or unpaired observations. All values are given as mean ± SEM.

RESULTS

Effects of cholinergic blockage

Subcutaneous administration of Pro-Banthine® resulted in a decreased protein output (Fig. 1) reflecting both a diminished protein concentration and a reduction in secretory volume. Thus, the hypersecretion of the bile-pancreatic juice in fistula rats was depressed by cholinergic blockage. Similar results were obtained in the experiments described in Fig. 2, which also shows that intraduodenal trypsin infusion did not significantly change bile-pancreatic output in rats given Pro-Banthine®. In the control group, trypsin infusion on the contrary inhibited the protein output. Furthermore, addition of trypsin inhibitor enhanced protein output in the control group. A moderate increase in protein output after addition of trypsin inhibitor occurred also in rats given Pro-Banthine®.

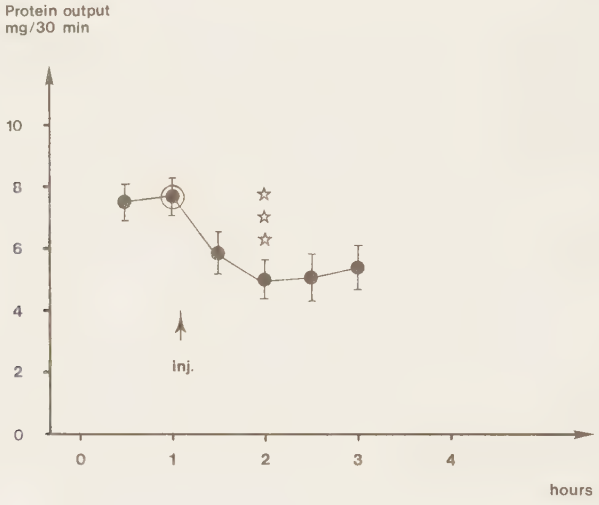


Fig. 1 Protein output in rats provided with bile-pancreatic fistulae (n=10). An anticholinergic agent (Pro-Banthine®; 4 mg/kg) was injected subcutaneously. Values are given as mean ± SEM. The circle indicates the pre-injection value used in the statistical calculation.
☆☆☆ = $p<0.001$

Effects of vagotomy

Following vagotomy (Fig. 3), a significant reduction of protein output, protein concentration and secretory volume was observed in rats provided with bile-pancreatic fistulae as compared to control fistula rats. These effects appeared even more pronounced after vagotomy than after cholinergic blockage. Contrary to in controls trypsin within the duodenum did not influence the secretion in vagotomized rats. Furthermore, in the vagotomized animals addition of trypsin inhibitor to the infusate did not cause an increased protein output (Fig. 3).

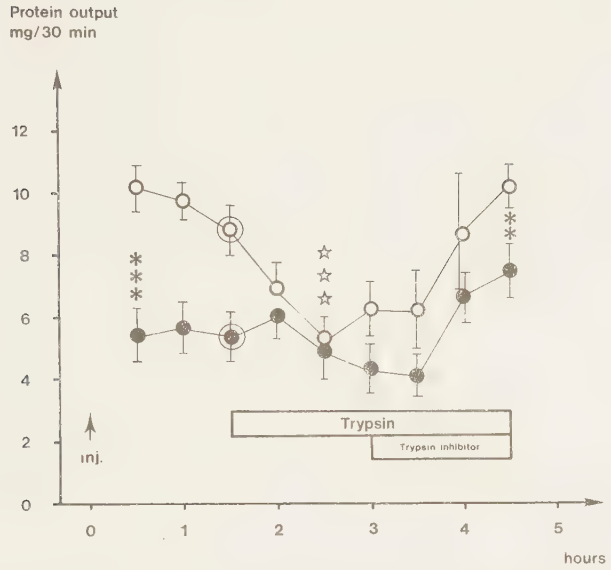


Fig. 2 Protein output in rats provided with bile-pancreatic fistulae. Trypsin (5 mg/ml 0.05 M $NaHCO_3$) was administered intraduodenally either without or with the addition of trypsin inhibitor. Eight rats (O—O) served as controls. Thirty minutes before the start of the experiment an anticholinergic agent (Pro-Banthine®; 4 mg/kg) was injected subcutaneously to eight rats (●—●). Values are given as mean ± SEM. The circles represent pre-infusion values. Probability levels of significant differences are indicated by (*) for differences between groups and by (☆) for differences related to preinfusion levels within the same group.
** = $p<0.01$ *** = $p<0.001$ ☆☆☆ = $p<0.001$

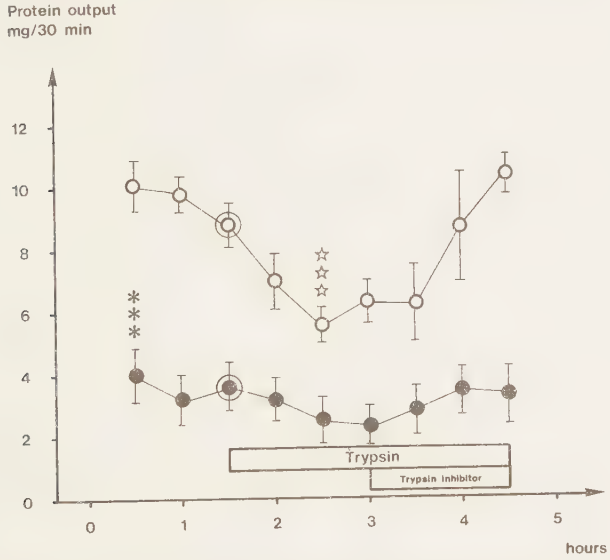


Fig. 3 Protein output in vagotomized rats (●—●; n=9) and in control rats (O—O; n=8) provided with bile-pancreatic fistulae. Trypsin (5 mg/ml 0.05 M $NaHCO_3$) was administered intraduodenally either without or with the addition of trypsin inhibitor. Values are given as mean ± SEM. The circles represent pre-infusion values. Probability levels of significant differences are indicated by (*) for differences between groups and by (☆) for differences related to preinfusion levels within the same group.
*** = $p<0.001$ ☆☆☆ = $p<0.001$

Effects of insulin hypoglycaemia

Vagal stimulation by means of hypoglycaemia did not further augment the protein secretion in fistula rats. Infusion of trypsin intraduodenally inhibited protein output both in the hypoglycaemic group and in the control group (Fig. 4). The inhibitory effect was slower in onset and occurred more gradually in the hypoglycaemic group than in the control group. After addition of trypsin inhibitor to the infusate the hypoglycaemic rats did not respond with increased secretion.

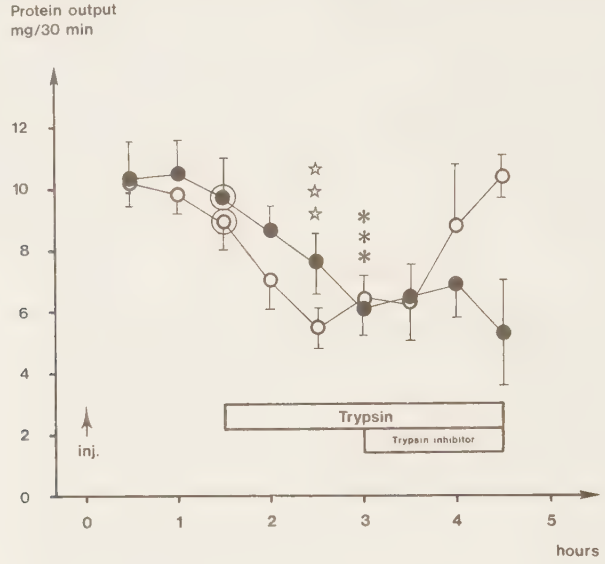


Fig. 4 Protein output in rats provided with bile-pancreatic fistulae. Trypsin (5 mg/ml 0.05 M $NaHCO_3$) was administered intraduodenally either without or with the addition of trypsin inhibitor. Eight rats served as controls (O—O). Hypoglycaemia was induced in eight rats (●—●) by insulin (1 U subcutaneously every second hour). The first insulin injection was given 30 minutes before the start of the experiment. Values are given as mean ± SEM. The circles represent pre-infusion values.
*** = $p<0.001$ ☆☆☆ = $p<0.001$

DISCUSSION

In the present study vagotomy as well as cholinergic blockage were shown to significantly depress the hypersecretion of protein from rats provided with bile-pancreatic duct fistulae. Furthermore, in contrast to in control rats intraduodenal trypsin infusion did not change the protein output after vagotomy or cholinergic blockage. Vagal stimulation induced by insulin hypoglymaemia failed to further stimulate the protein output in rats with their bile-pancreatic juice deviated. In these latter rats, however, intraduodenal infusion of trypsin markedly inhibited protein secretion.

In the presence of bile-pancreatic juice or trypsin within the duodenum additional infusion of trypsin inhibitor causes an immediate stimulation of the pancreatic enzyme secretion (18). It is also known that long-term oral administration of naturally occurring or purified trypsin inhibitors

exerts a trophic effect on the pancreas with increased activities of digestive enzymes (1). In a previous study we found that long-term parenteral trypsin inhibitor administration in the rat did not result in changes of pancreatic morphology or enzyme activities (14). These findings suggest that the intestinal mucosa is involved in the mediation of the trypsin inhibitor effects on the pancreas. We have also demonstrated that resection of the proximal third of the small intestine in the rat abolished the effects of oral long-term administration of trypsin inhibitors (13). These findings indirectly propose a hormonal mediation of the trypsin inhibitor effects. We also observed that CCK-PZ as well as the octapeptide of CCK-PZ resulted in similar - but not identical - changes of exocrine pancreatic weight and enzyme activities as did long-term oral trypsin inhibitor ingestion (15). Futhermore, in normal as well as in diabetic rats trypsin inhibitor ingestion for 5-7 weeks significantly improved glucose tolerance mainly as a result of increased glucose utilization in peripheral tissues (23). All these data suggest that gastrointestinal hormones or factors are responsible for the pancreatic effects found after long-term trypsin inhibitor ingestion in the rat.

In acute experiments Schneeman et al. (27) demonstrated that trypsin in the proximal part of the intestine was inhibitory to pancreatic enzyme secretion, while trypsin infused into the distal part of the intestine was without effect. These results are in accordance with a hormonal mediation from the upper intestine. Further evidence for blood born factor(s) in this context was provided by Khayambashi and Lyman (19) who reported that when plasma from rats fed trypsin inhibitor was perfused through an isolated fasted rat pancreas the amylase secretion was increased three times that of the pancreas perfused with plasma from rats fed with the same diet without trypsin inhibitor. Using isolated exocrine pancreatic cells in suspension Toregard et al. (29) also reported on more direct evidence for a humoral factor in plasma from trypsin inhibitor fed rats causing increased lipase and amylase secretion from the cells. On the other hand Lyman et al. (25) in an early study did not find any evidence for the release of a humoral factor by trypsin inhibitor administration in cross-circulation experiments in the rat.

Practically no attention has been paid to a possible neural involvement in the trypsin-induced feed-back regulation of pancreatic secretion. Petersen and Grossman (26) recently reported that the hypersecretion induced by deviation of bile-pancreatic juice and the inhibition of pancreatic secretion following re-infusion of bile-pancreatic juice into the intestine were unaffected by vagotomy. These results are in contrast to our present findings of decreased protein output via the mixed bile-pancreatic juice in vagotomized rats. In conformity with our findings the endogenous stimulation of pancreatic secretion in dog and man has been reported to be diminished after vagotomy (11, 30). Our data furthermore suggest that vagal integrity is of importance for the feed-back regulation of pancreatic secretion exerted by intraluminal trypsin. As stated above this regulation probably is mediated via humoral factors. The intimate mechanisms in this neuro-humoral interaction await further elucidation.

In agreement with the findings after vagotomy we also found that cholinergic blockage decreased protein output in bile-pancreatic fistula rats and abolished the trypsin-induced feed-back regulation of the bile-pancreatic protein output. The effects of this pharmacological blockage were less pronounced than after complete surgical vagotomy. Grossman was unable to demonstrate an inhibitory effect on pancreatic protein secretion by atropine in fistula rats (9). In the dog Konturek (20) found that atropine diminished pancreatic flow and bicarbonate output in response to exogenous as well as endogenous stimulation by secretin. Furthermore atropine in their studies caused a marked and prolonged decrease in pancreatic flow rate and protein and bicarbonate output in response to release of CCK-PZ from the intestinal mucosa. Also in man administration of anticholinergic drugs are known to reduce pancreatic volume response by 23-43 % during CCK-PZ/secretin tests (2). Thus, most studies - supporting the results of the present study - demonstrated that cholinergic blockage reduced pancreatic enzyme output.

Indirect vagal stimulation by 2-deoxyglucose and by insulin hypoglycaemia has been shown to stimulate pancreatic enzyme secretion (3). We did not find any further increase of the protein output during insulin hypoglycaemia in rats with bile-pancreatic fistulae. In these rats - as in the controls - intraduodenal trypsin infusion depressed the protein output, although the response was slower in onset and more gradual than in the control rats. Addition of trypsin inhibitor to the trypsin infusate, however, failed to stimulate the pancreatic secretion in the hypoglycaemic rats. This might partly reflect exhaustion of the exocrine pancreatic cells following the interaction of hormonal and neural stimulation during the first part of the experiment but might also be a consequence of e.g. the hyperglucagonaemia seen during hypoglycaemia (5).

The results of the present study re-emphasize the close interaction between the autonomic nervous system and gastrointestinal hormones on pancreatic protein secretion. Considering the results of the vagotomy and cholinergic blockage experiments it seems as if vagal integrity - at least in acute experiments - is important to the feed-back regulation of pancreatic enzyme secretion exerted by intraluminal trypsin in the rat.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Medical Research Council (Projects No 17X-03012 and 04P-4289), the Medical Faculty of Lund, the Albert Påhlsson Foundation and the Greta and Harald Jeansson Foundation.

REFERENCES

1. Arnesjö, B., Ihse, I., Lundquist, I. and Quist, I.
Scand. J. Gastroent. 1973, 8, 545-554

2. Bock, O.A.A., Bank, S., Marks, I.N. et al.
Gastroenterology, 1968, 55, 199-203

3. Brown, J.C., Harper, A.A. and Scratcherd, T.
J. Physiol. (Lond.) 1971, 216, 303-318

4. Chernik, S.S., Lepkovsky, S. and Chaikoff, I.L.
Am. J. Physiol. 1948, 155, 33-41

5. Dyck, W.P., Rudick, J., Hoexter, B. et al.
Gastroenterology 1969, 56, 531-537

6. Eggstein, M. and Creutz, F.M.
Klin. Wschr. 1955, 33, 879-884

7. Frey, E.K., Kraut, H. and Werle, E.
Das Kallikrein-Kinin-System unde seine Inhibitionen. Ferdinand Enke Verlag, Stuttgart, 1967

8. Green, G.M. and Lyman, R.L.
Proc. Soc. Exp. Biol. Med. 1972, 140, 6-12

9. Grossman, M.I.
Am. J. Physiol. 1958, 194 (3), 535-539

10. Håkanson, R., Alumets, J., Ekelund, M. et al.
Scand. J. Gastroent. 1978, suppl. 55, p. 24

11. Henriksen, F.W.
Scand. J. Gastroent. 1969, 4, 137-142

12. Hummel, B.W.C.
Canad. J. Biochem. Biophys. 1959, 37, 1393-1399

13. Ihse, I.
Scand. J. Gastroent. 1976, 11, 11-17

14. Ihse, I., Arnesjö, B. and Lundquist, I.
Scand. J. Gastroent. 1974, 9, 719-724

15. Ihse, I., Arnesjö, B. and Lundquist, I.
Scand. J. Gastroent. 1976, 11, 529-535

16. Ihse, I. and Lilja, P.
Scand. J. Gastroent. In press.

17. Ihse, I., Lilja, P. and Lundquist, I.
Digestion 1977, 15, 303-308

18. Ihse, I., Lilja, P. and Lundquist, I.
Scand. J. Gastroent. In press.

19. Khayambashi, H. and Lyman, R.L.
Am. J. Physiol. 1969, 217, 646-651

20. Konturek, S.J., Tasler, J. and Obtulowicz, L
Am. J. Dig. Dis. 1971, 16, 385-394

21. Lilja, P. and Ihse, I.
Danish Med. Bull. 1979, 26, suppl. 1, p. 12

22. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J.
J. Biol. Chem. 1951, 193, 265-271

23. Lundquist, I., Ihse, I. and Arnesjö, B.
Scand. J. Gastroent. 1976, 11, 369-375

24. Lyman, R.L. and Lepkovsky, S.
J. Nutr. 1957, 62, 269-284

25. Lyman, R.L., Wilcox, S.S. and Monsen, E.R.
Am. J. Physiol. 1962, 202 (6), 1077-1082

26. Petersen, H. and Grossman, M.J.
Am. J. Physiol. 1977, 233 (6), E530-E536

27. Schneeman, B.O. and Lyman, R.L.
Proc. Socl. Exp. Biol. Med. 1975, 148, 897-903

28. Shay, H., Komarov, S.A. and Gruenstein, M.
Arch. Surg. 1949, 59, 210-215

29. Toregard, B.M., Borgström, B., Ihse, I. and Nilsson, A.
Danish Med. Bull. 1979, 26, suppl. 1, p. 6

30. Wormsley, K.G.
Scand. J. Gastroent. 1972, 7, 85-91

EFFECTS OF INTESTINAL AMYLASE AND TRYPSIN ON PANCREATIC SECRETION IN THE PIG

Ingemar Ihse and Per Lilja

Department of Surgery, University of Lund,

S-221 85 Lund, Sweden

Abstract

Pigs were surgically prepared with external pancreatic fistulae and duodenal cannulae in order to elucidate whether the proposed intestinal feedback control of pancreatic secretion in the pig - as in rat and man - is exerted by trypsin. Furthermore the effect of intraluminal amylase on pancreatic secretion was studied. Reintroduction of pancreatic juice into the duodenum or infusion into the duodenum of trypsin depressed the volume of the pancreatic flow and the protein output markedly. Introduction of amylase into the duodenum did not significantly affect the pancreatic secretion. Thus, it seemed as if trypsin but not amylase was involved in the suppression exerted by pancreatic juice on exocrine pancreatic secretion in the pig.

Key-words: Amylase; Feedback regulation; Pancreatic juice; Pancreatic secretion; Pig; Trypsin.

Reprtint requests: Per Lilja, M.D., Department of Surgery, University of Lund, S-221 85 Lund, Sweden.

It is well established that long term peroral administration of trypsin inhibitor to rats and chickens results in morphological and functional changes of the pancreas (1,2). These findings speak in favour of a relationship between intraduodenal tryptic activity and pancreatic secretion. In acute experiments in rat a feedback regulation of pancreatic secretion exerted - at least partly - by intraluminal trypsin has been shown (7,13). Amylase and lipase, however, did not influence pancreatic secretion (14). Magee and Hong (18) and Corring (3) reported inhibition of pancreatic enzyme secretion by reintroduction of pancreatic juice into the small intestine of pigs provided with external pancreatic fistulae. In a previous study we furthermore found evidence for the existence of a feedback regulation of pancreatic secretion in man (12).

To our knowledge no experimental data beside those by Corring (3) have been reported on which intraduodenal components of the pancreatic juice that influence pancreatic secretion in the pig. Therefore the present investigation was undertaken to elucidate whether the proposed feedback control of pancreatic secretion in the pig - as in rat and man - is exerted by trypsin. Furthermore the effect of intraduodenal amylase on pancreatic secretion was studied.

Methods

Animals. Male pigs weighing 15 kg were anesthetized with fluothane, N_2O and O_2. Via a midline abdominal incision the duodenum was exposed and the pancreatic duct cannulated with a baby feeding tube (outer diameter 3.5 mm) and divided at the point of entry into the intestine. A duodenal cannula was established by inserting a Silastic tube through an incision in the intestinal wall 10 cm distal to the pylorus. The two cannulae were brought out through separate abdominal wall incisions (fig. 1).

The animals were allowed 48 hours of surgical recovery before the experiments were started. During this period the pancreatic juice was returned into the intestine by joining the pancreatic duct cannula and the duodenal cannula. The experiments were performed in restraining cages after 8 hours of fasting.

During the experiments an infusion of isotonic 5.5% glucose-solution with the addition of 80 mmol NaCl were administered intravenously at a rate of 50 ml per hour.

Duodenal infusates. An infusion pump was used and all infusates were administered at a constant rate of 1.5 ml per minute which corresponded to double the volume secreted from the pancreas. The pH of the infusates was corrected to 8.0 ± 0.2. The temperature of the infusates was brought to 25°C immediately before introduction into the duodenum.

Pancreatic juice for intraduodenal infusion was collected on ice the day before the experiments and frozen at -20°C.

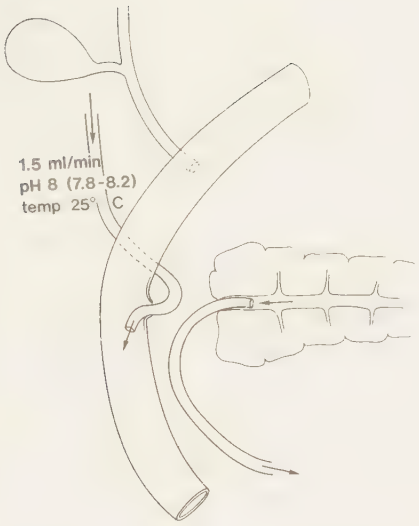


Fig. 1 The preparation in pigs. Cannulae were inserted into the pancreatic duct and the duodenum, respectively. Rate, pH and temperature of the infusates were kept constant during administration.

Porcine crystalline trypsin was obtained from A/S Novo, Copenhagen, Denmark. It was dissolved in 0.05 M $NaHCO_3$ to a concentration of 1 mg/ml. The tryptic activity of this solution was 140 U/ml as determined by a modified Hummel method using TAME (þ-toluene-sulphonyl-L-arginine methyl ester) as the substrate (9). This activity roughly corresponded to the normal tryptic activity in the duodenum of the pig.

Crystalline porcine amylase was obtained from Sigma Ltd, S:t Louis, U S A. It was dissolved in 0.05 M $NaHCO_3$ to a concentration of 50 mg/ml. The amylolytic activity of this solution was 500 U/ml determined as described by Dahlqvist (4) using maltose as the substrate. The activity roughly corresponded to the normal intraduodenal amylolytic activity in the pig. In ancillary experiments the amylase preparation was found to be slightly polluted with trypsin(ogen) (13).

Highly purified bovine lung trypsin inhibitor was obtained from Bayer AG, Leverkusen, Germany. This inhibitor is not absorbed from the intestine (20) or digested by intestinal proteolytic enzymes and inhibits trypsin at a pH optimum of 8 (6). Ancillary experiments in our laboratory showed that 0.5 mg of the inhibitor completely abolished the tryptic activity of 1 mg of the trypsin used.

Evaluation of pancreatic secretion. Pancreatic juice was collected from the pancreatic fistulae in tubes on ice at 90 minute intervals and immediately frozen at -20°C until analyzed within one week. The volume of the samples of pancreatic juice was measured by weight and the concentration of protein estimated in duplicate according to the method

described by Lowry et al (15) as modified by Eggstein and Creutz (5).

Calculations. The data obtained were subjected to statistical analysis using paired Student's t-test. All values are given as mean ± SEM. Differences were considered significant at p-values below 0.05.

RESULTS

Reintroduction of pancreatic juice into the duodenum. The spontaneous pancreatic secretion with diverted pancreatic juice varied markedly between the animals but only slightly in repeated samples from the same animal. The infusion of 1.5 ml/minute of 0.05 M $NaHCO_3$ into the duodenum did not change the pancreatic secretory volume or protein output (data not shown). Reintroduction, however, of pancreatic juice into the duodenum depressed the pancreatic secretory volume ($p < 0.01$) and the pancreatic protein output ($p < 0.05$) significantly in the experimental model used (fig. 2).

Infusion of trypsin into the duodenum. This experiment was designed to elucidate the effects of intraduodenal trypsin on the pancreatic secretion. Infusion (1.5 ml/min) into the duodenum of trypsin dissolved in 0.05 M $NaHCO_3$ (1 mg/ml) suppressed the pancreatic secretory volume significantly ($p < 0.05$). Furthermore the pancreatic protein output was markedly reduced ($p < 0.05$) (fig. 3).

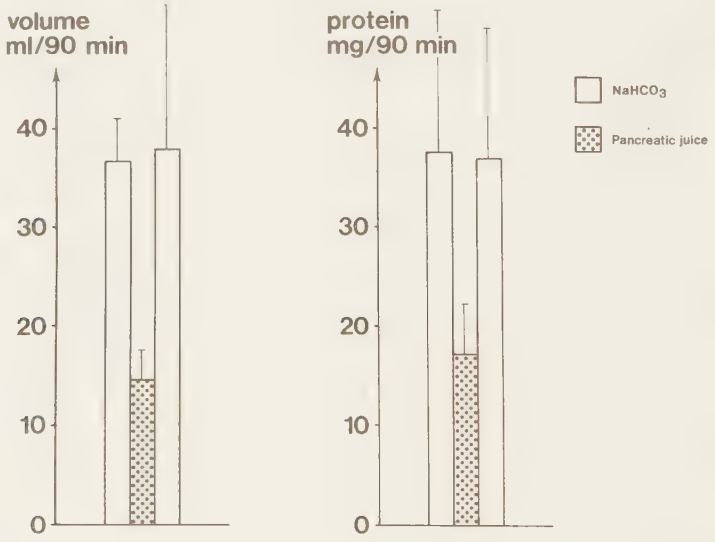


Fig. 2 Pancreatic secretory volume and protein output in pigs provided with pancreatic fistulae. 1.5 ml/min of 0.05 M $NaHCO_3$ and pancreatic juice, respectively, were infused intraduodenally. Values are given as mean ± SEM. N = 4. The secretory volume and the protein output decreased significantly ($p < 0.01$ and $p < 0.05$, respectively).

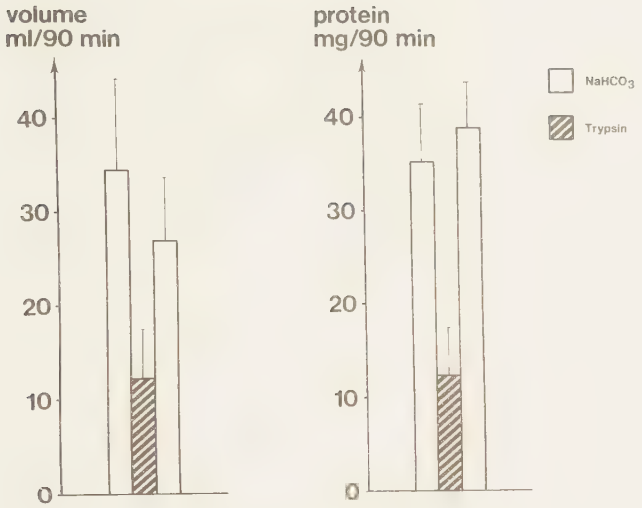


Fig. 3 Pancreatic secretory volume and protein output in pigs provided with pancreatic fistulae. 1.5 ml/min of 0.05 M $NaHCO_3$ and trypsin (1 mg/ml 0.05 M $NaHCO_3$), respectively, were infused intraduodenally. Values are given as mean ± SEM. N = 4. The secretory volume and protein output decreased significantly ($p < 0.05$).

Infusion of amylase into the duodenum. In this experiment porcine amylase dissolved in 0.05 M $NaHCO_3$ (50 mg/ml) was infused intraduodenally (1.5 ml/min) in order to study if this enzyme exerted any effects on pancreatic secretion. As can be seen in fig. 4 no significant changes of pancreatic secretory volume or protein output was observed following amylase infusion.

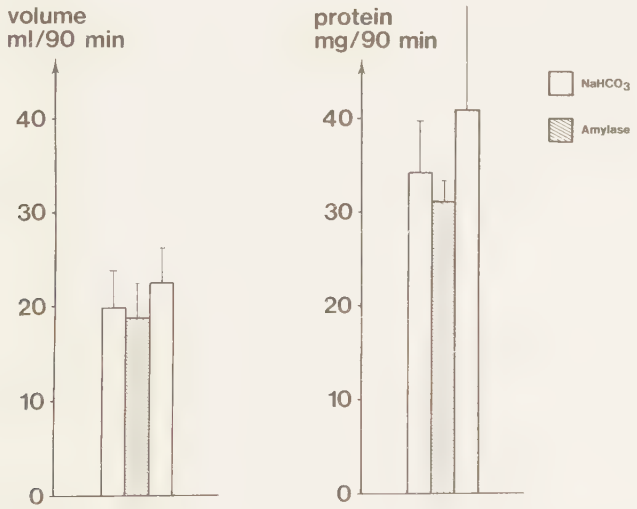


Fig. 4 Pancreatic secretory volume and protein output in pigs provided with pancreatic fistulae. 1.5 ml/min of 0.05 M $NaHCO_3$ and amylase (50 mg/ml 0.05 M $NaHCO_3$), respectively, were infused intraduodenally. Values are given as mean ± SEM. N = 4.

Reintroduction of pancreatic juice without and with addition of trypsin inhibitor. This experiment (a single experiment) was designed to study whether the suppression of pancreatic secretion caused by reinfusion of pancreatic juice into the duodenum could be abolished by the addition of trypsin inhibitor to the infusate. As can be seen from fig. 5 the inhibitory effect on the pancreatic secretory volume and protein output induced by intraduodenal pancreatic juice was reversed when trypsin inhibitor was added to the infusate in amounts sufficient to eliminate all intraduodenal tryptic activity.

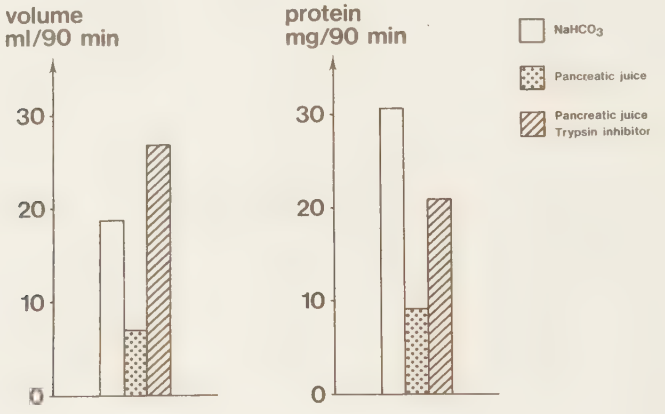


Fig. 5 Pancreatic secretory volume and protein output in one pig provided with a pancreatic fistula. 1.5 ml/min of 0.05 M $NaHCO_3$, pancreatic juice and pancreatic juice with trypsin inhibitor, respectively, were infused intraduodenally.

Discussion

In the present study in conscious pigs surgically prepared with pancreatic fistulae reintroduction of pancreatic juice into the duodenum suppressed pancreatic secretory volume and protein output. Furthermore it was found that intraduodenal trypsin in contrast to amylase exerted such a suppression. In a single experiment the introduction of trypsin inhibitor intraduodenally reversed the inhibitory effects on pancreatic secretion induced by pancreatic juice.

This study confirms and extends the result reported by Magee and Hong (18), Hong et al (8) and Corring (3) who found increased pancreatic secretion in the pig after excluding pancreatic juice from the duodenum and a subsequent inhibition when the juice was reintroduced. Magee and Hong (18), however, suggested that the depression observed after reintroduction of pancreatic juice was a result of increased intraduodenal pH. Our study does not support this hypothesis but rather points to other mechanisms in the regulation of pancreatic secretion (vide infra).

Previously we have demonstrated in the rat that reintroduction of bile-pancreatic juice suppressed pancreatic protein output mainly by changing the concentration of proteins rather than the secretory volume (13). In man, however, we found that intraluminal pancreatic juice influenced the pancreatic secretory volume more than the protein concentration (12). In this study in pigs it seemed as if the pancreatic secretion was influenced by alterations of the volume rather than of concentration of proteins. If these findings reflect species differences or if they suggest different mediators operating in the regulation of pancreatic secretion cannot be judged at the moment.

Our data is in conformity with those of Corring (3) that trypsin within the duodenum is involved in the feedback regulation of pancreatic secretion in the pig. Infusion of trypsin and reintroduction of pancreatic juice depressed pancreatic secretion to about the same extent. Furthermore in a single experiment we found that in the presence of pancreatic juice intraduodenal infusion of trypsin inhibitor caused a marked stimulation of the pancreatic flow. No conclusion can be drawn from this single experiment but our finding is consistent with what we have demonstrated in the rat (13) and in man (12).

Amylas, infused into the duodenum, did not influence the pancreatic secretion significantly. However, a minimal depression of the mean values was observed (fig. 4). This finding might be due to a slight tryptic contamination of the amylase preparation used. Macri et al (17) found pancreatic hypertrophy and an increased amylase production in chickens fed with albumin-amylase inhibitors speaking in favour of a relationship between duodenal amylolytic activity and pancreatic secretion. However, their amylase inhibitor contained components active in inhibiting trypsin as well (17) and their findings were similar to those reported on feeding of trypsin inhibitors (1,2).

The mechanism behind the suggested feedback regulation of pancreatic secretion is not fully understood. It has been suggested that trypsin inhibitor induced pancreatic secretion is mediated by release of one or more intestinal factors or hormones (16). In previous studies we have shown that long-term parenteral trypsin inhibitor administration in the rat did not induce pancreatic hypertrophy in contrast to oral administration (11). Furthermore we have demonstrated that resection of the proximal third of the small intestine abolished the effects on the pancreas induced by oral trypsin inhibitor administration (10). As most gastrointestinal hormones are localised to the upper third of the gut (19) our previous experiments speak in favour of a hormonal mediation of the pancreatic feedback regulation. Hitherto, however, it is not established which hormone that is the main mediator.

In summary, the present paper shows that in pigs surgically prepared with external pancreatic fistulae and duodenal cannulae intraduodenal infusion of trypsin as well as reintroduction of pancreatic juice inhibited pancreatic secretory volume and protein output. Furthermore it was demonstrated that intraduodenal administration of amylase was without effect on the pancreatic secretory volume and protein output. Thus it seems as if trypsin in contrast to amylase in the pig is involved in the suppression of exocrine pancreatic secretion exerted by pancreatic juice in the duodenum.

References

1. Arnesjö, B., Ihse, I., Lundqvist, I. & Qvist, I.
   Scand. J. Gastroent. 1973, 8, 545-554

2. Chernik, S.S., Lepkovsky, S. & Chaikoff, I.L.
   Amer. J. Physiol. 1948, 155, 33-41

3. Corring, T.
   Ann. Biol. Anim. Biochim. Biophys. 1974, 14, 487-498

4. Dahlqvist, A.
   Scand. J. Clin. Lab. Invest. 1962, 14, 145-151

5. Eggstein, M. & Creutz, F.M.
   Klin. Wschr. 1955, 33, 879-884

6. Frey, E.K., Kraut, H. & Werle, E.
   Das Kallikrein-Kinin System und seine Inhibitoren.
   Ferdinand Enke Verlag, Stuttgart, 1967

7. Green, G.M. & Lyman, R.L.
   Proc. Soc. Exp. Biol. Med. 1972, 140, 6-12

8. Hong, S.S., Nakamura, M. & Magee, D.F.
   Ann. Surg. 1967, 778-782

9. Hummel, B.W.C.
Canad. J. Biochem. Piophys. 1959, 37, 1393-1399

10. Ihse, I.
Scand. J. Gastroent. 1976, 11, 11-17

11. Ihse, I., Arnesjö, B. & Lundquist, I.
Scand. J. Gastroent. 1974, 9, 719-724

12. Ihse, I., Lilja, P. & Lundquist, I.
Digestion, 1977, 15, 303-308

13. Ihse, I., Lilja, P. & Lundquist, I.
Scand. J. Gastroent. In press.

14. Lilja, P.
To be published.

15. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. & Randall, R.J.
J. Biol. Chem. 1951, 193, 265-271

16. Lyman, R.L. & Lepkovsky, S.J.
Nutr. 1957, 62, 269-284

17. Macri, A., Parlamenti, R., Silano, V. & Valfree, F.
Poultry Science 1977, 56, 434-441

18. Magee, D.F. & Hong, S.S.
Swine in Biochemical Research 1965, 109-119

19. Schneeman, B.O. & Lyman, R.L.
Digestion 1977, 15, 28-33

20. Wilson, P.A., Melmed, R.N., Hampe, M.M.V. & Holt, S.J.
Gut 1978, 19, 260-266

Accepted for publication in Scandinavian Journal of Gastroenterology.

INTESTINAL CONCENTRATIONS OF PANCREATIC ENZYMES FOLLOWING PANCREATIC REPLACEMENT THERAPY

I. Ihse, P. Lilja and I. Lundquist

Departments of Surgery and Pharmacology, University of Lund, Lund, Sweden.

Abstract

The activities of amylase, lipase, phospholipase and trypsin of four commercially available preparations of pancreatin with different galenic and adjunctive protective properties were estimated *in vitro* using human small intestinal juice as the incubation medium. These preparations were administered to healthy subjects and to patients with severe pancreatic insufficiency and their ability to increase the intestinal concentrations of pancreatic enzyme was evaluated. The relations between *in vitro* and *in vivo* activities were also studied. *In vitro* testing showed that the preparations contained high but varying activities of enzymes with the greatest variations in lipase and trypsin. Pancreatin in the form of tablets, with or without protective measures against acid, did not cause any apparent increase of the activities of pancreatic enzymes in the upper part of the gut in patients with pancreatic insufficiency. Granulated pancreatin on the other hand brought about an increase of the activities of amylase, phospholipase, lipase and trypsin. Relatively higher activities of the enzymes in granulated form reached the small intestine as compared to those of the tablets.

Key-words: Intestinal enzyme concentrations;
Pancreatin; Pancreatic enzymes;
Pancreatic insufficiency;
Replacement therapy

Reprint requests: Per Lilja, M.D., Department of Surgery,
University of Lund, S-221 85 Lund, Sweden.

Pancreatic replacement therapy is used in malabsorption due to pancreatic insufficiency. Although secondary objectives such as weight gain and sense of well-being often is accomplished, steatorrhea is seldom completely restored. The main goal of the treatment is to get sufficient amount of active enzymes to the duodenum at the time when food is passing into the small bowel. Until recently, however, no information has been available on the ability of pancreatic enzyme replacements to change the intestinal concentrations of these enzymes. In 1977 di Magno et al. (5) found in a small number of patients that only 22 per cent of trypsin and 8 per cent of lipase administered reached the ligament of Treitz in active form. After pancreatin administration the trypsin at its maximum reached 3 per cent and lipase 1 per cent of the mean concentration in healthy subjects.

Its is well established that there is a steep, inverse relation between the degree of steatorrhea and azotorrhea and estimated trypsin and lipase activities in the duodenum (6,15). In the present investigation the effect of oral administration of 4 galenically different preparations of pancreatin on intestinal concentrations of amylase, lipase, phospholipase and trypsin was studied. Additionally the relation between *in vitro* and *in vivo* activities of the different preparations was elucidated.

MATERIAL AND METHODS

Pancreatic enzyme preparations

Four commercially available preparations of pancreatin with different galenic and adjunctive protective properties were used.

One preparation was a simple tablet without adjunctive protective properties (Pancreozym^{R}, TIKA, Sweden); one a tablet with tannin-bound enzymes, which by the manufacturer is claimed to be a protection against low pH and pepsin (Pancreon forte^{R}, Kali-Chemie, West Germany); one an enteric-coated granulated preparation (Pancreatin granules^{R}, Rosco, Denmark); and one a granulated remedy with tannin-binding (Pancreon granules^{R}, Kali-Chemie, West Germany). All preparations were distributed by the hospital pharmacy.

In vitro studies

One tablet or 10 ml of the preparations in granulated form was crushed in a mortar and incubated for 40 minutes at a temperature of 37°C in 50 ml pooled human duodenal juice obtained from normal subjects. Six tablets or 6 10 ml-dosages from two separate batches of each preparation were analyzed. The activities of amylase, lipase, phospholipase and trypsin of the incubation medium (human duodenal juice) were estimated before and after incubation with the pancreatin preparation. Thus the enzyme activities of the different preparations were calculated as the activities estimated after incubation minus those estimated before addition of the pancreatin preparations to the incubation medium.

Subjects

Nine healthy students and 24 patients with verified severe exocrine pancreatic insufficiency were studied.

Ten of the 24 patients suffered from chronic pancreatitis; in 8 the diagnosis was non-operated pancreatic cancer and in 6 total pancreatectomy had been performed prior to the investigation. The patients were randomly allocated into four groups with six in each. The plan of the study was accepted by the Ethical Comitté of the hospital.

In vivo studies

The patients and the healthy subjects were investigated after 12 hours fasting without premedication. A duodenal tube (Levine nr 12) was placed in the horizontal part of the duodenal loop under fluoroscopic control with the tip at the ligamentum of Treitz. In patients who had undergone total pancreatectomy the tip of the tube was positioned 40 cm below the gastro-jejunostomy.

A test meal according to Lundh (16) either without or with a pancreatin preparation was given to each subject at two different days in random order. Double recommended dosages - 8 tablets of Pancreozym^{R} (6 patients), 4 tablets of Pancreon forte^{R} (6 patients), 20 millilitres of Pancreatin granules^{R} (6 patients) or Pancreon granules^{R} (6 patients) - were administered together with the test meal. The sampling was started immediately after the administration of the meal. During the first hour the duodenal aspirates were sampled in 10-minute fractions and during the second hour in 30-minute fractions. From each sample 1 ml was withdrawn after shaking for 1-2 minutes and pooled. All collections were made under ice and stored at -20^{o}C until analyzed. The enzyme determinations were done in duplicate and only on the pooled aspirates.

Determination of enzymic activities

Amylase was determined according to Dahlqvist (4) and lipase by a scaled-down version of the method described by Erlanson & Borgström (8). Phospholipase was determined according to Ihse and Arnesjö (13) and trypsin as described by Hummel using TAME (p-toluene sulphonyl-L-arginine methyl ester) as the substrate (12).

In the in vitro studies no activators for prophospholipase and trypsinogen were needed as human small intestinal juice was chosen as incubation medium.

Calculations

Data obtained were subjected to statistical analysis using unpaired or paired Student's *t*-test.

RESULTS

In vitro studies

The estimated enzyme activities of pancreatin were independent of the duration of incubation at the time intervals studied (10, 25, 40 and 55 minutes, respectively). In the present study an incubation time of 40 minutes was chosen.

In Table I the enzyme content of the different preparations is presented. There were wide variations of the activities of most enzymes between batch I and II of each preparation. Within each batch, however, the variations ot the enzyme activities were small. It is obvious from the results (Table I) that the enzyme content varied markedly also between one tablet of Pancreozym^{R} and one tablet of Pancreon forte^{R}. On the other hand there was a better agreement of the enzyme content of equivalent doses of the two granulated preparations.

As the amount of pancreatin of each tablet or mg of granules varied markedly, as described by the manufacturers, we calculated the activity of the enzymes per gram pancreatin (Fig. 1). The preparations contained high but varying activities also per gram pancreatin with the greatest variations in lipase and trypsin. Further the preparations in granulated form seemed to exert lower enzyme activities per gram pancreatin than the tablets. The lipase and phospholipase activity per gram pancreatin was highest in the preparation which was manufactured in the less complex way (Pancreozym^{R}).

In vivo studies

Pancreatin in the form of a simple tablet (Pancreozym^{R}) when given to 12 healthy persons in double recommended dose did not cause a significant increase of either amylase, lipase, phospholipase or trypsin in the duodenum (data not shown). Furthermore this preparation did not significantly affect the enzyme concentrations in patients suffering from pancreatic insufficiency (Fig. 2 A). Neither did pancreatin in the form of a tablet with tannin-binding as protection against acid (Pancreon forte^{R}) cause an increase of the activities of pancreatic enzymes in the upper part of the gut in patients with pancreatic insufficiency (Fig. 2 B).

The two preparations in granulated form, however, both with protective mechanisms against acid, did bring about an increase of the activities of amylase, phospholipase, trypsin and lipase (Fig. 2 C-D). The granulated Rosco preparation did actually normalize the intraduodenal concentrations of amylase, phospholipase and trypsin while the increase of lipase was only about 20 per cent. The granulated Pancreon preparation normalized the concentrations of amylase and brought about a two-fold increase of lipase and three-fold increase of phospholipase and trypsin.

Table I. Mean enzyme activities ± S.E.M. of 6 tablets or 6 doses of granulated preparations from two different batches. Further mean enzyme activities of the doses of the various preparations administered to the human subjects in the in vivo study is given. T = Tablet; G = Granules.

	Pancreozym (T)	Pancreon forte (T)	Pancreatin granules (G)	Pancreon granules (G)
AMYLASE				
Units per tablet or 5 ml of granules				
Batch I + II	5 909±565	24 550±567	52 511±7080	35 457±1754
Units per dose given	46 272	98 200	210 044	141 828
LIPASE				
Units per tablet or 5 ml of granules				
Batch I + II	4 035±702	11 861±2883	8 187±394	9 334±1098
Units per dose given	32 280	47 444	34 748	37 336
PHOSPHOLIPASE				
Units per tablet or 5 ml of granules				
Batch I + II	497±89	1 308±240	2 747±45	2 411±197
Units per dose given	3 975	5 232	10 985	9 644
TRYPSIN				
Units per tablet or 5 ml of granules				
Batch I + II	1 288±218	4 574±375	4 383±55	5 644±604
Units per dose given	10 304	18 295	17 532	20 175

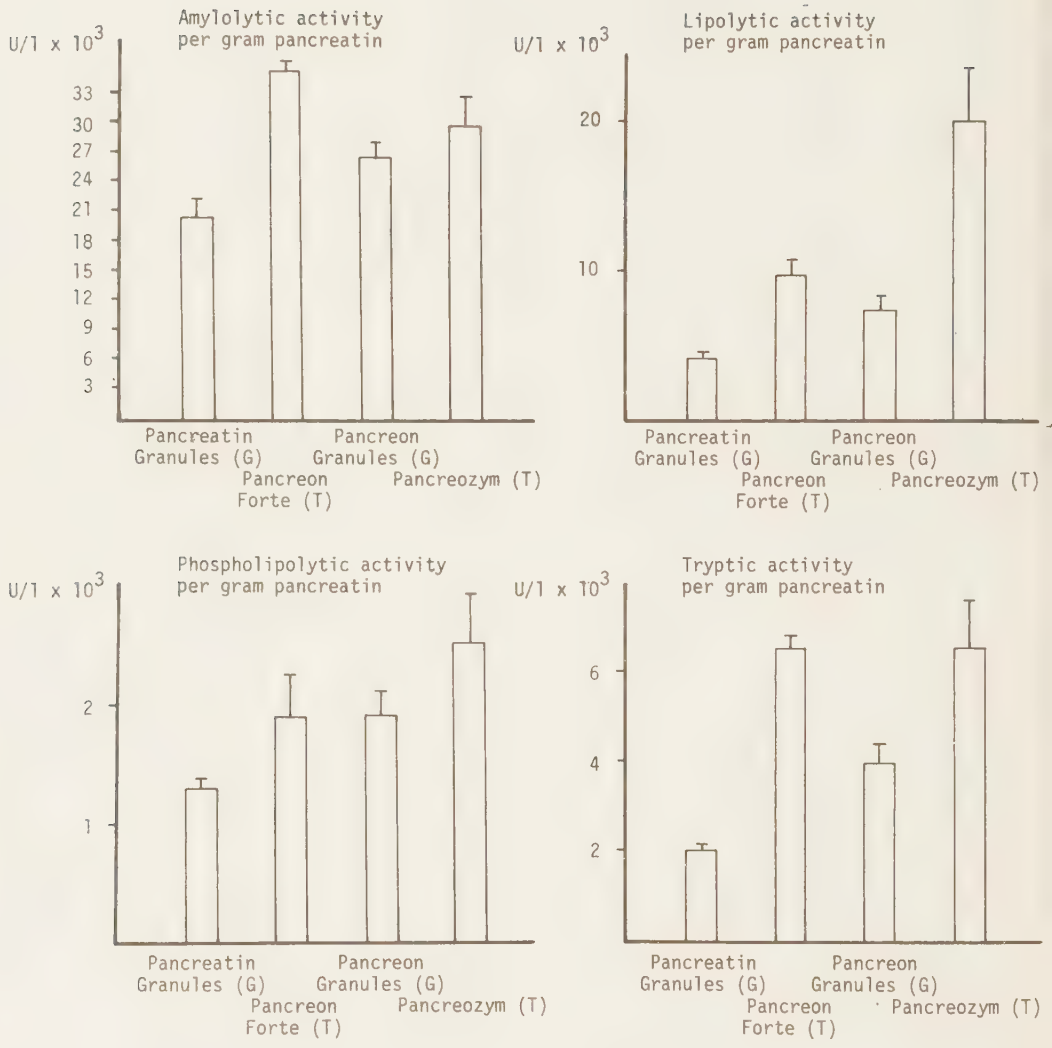
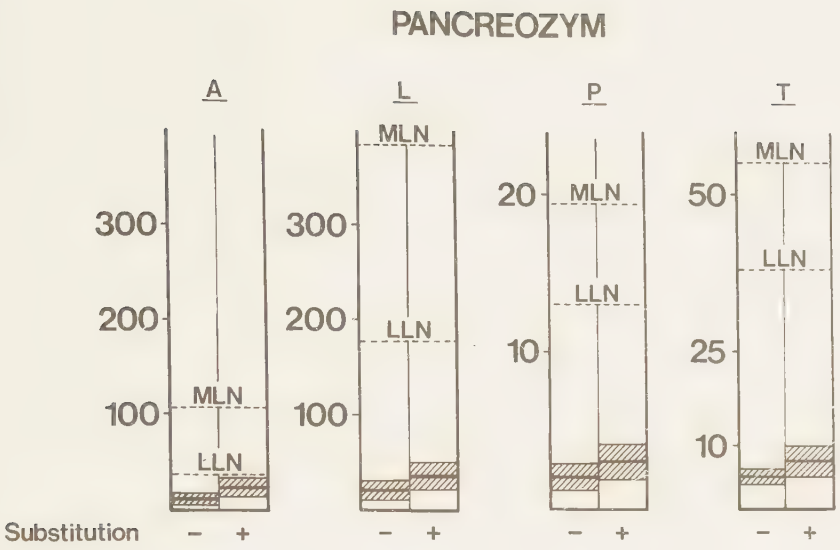


Fig. 1 Enzymic activity per gram pancreatin in four different supplements. T = tablets, G = granules. The values are given as mean ± S.E.M. Amylase activity is expressed as µmole maltose released per ml per min. Lipase activity is expressed as µmole fatty acids released per ml per min. Phospholipase activity is expressed as µmole fatty acid released per ml per min. Trypsin activity is expressed as µmole TAME hydrolyzed per ml per min.



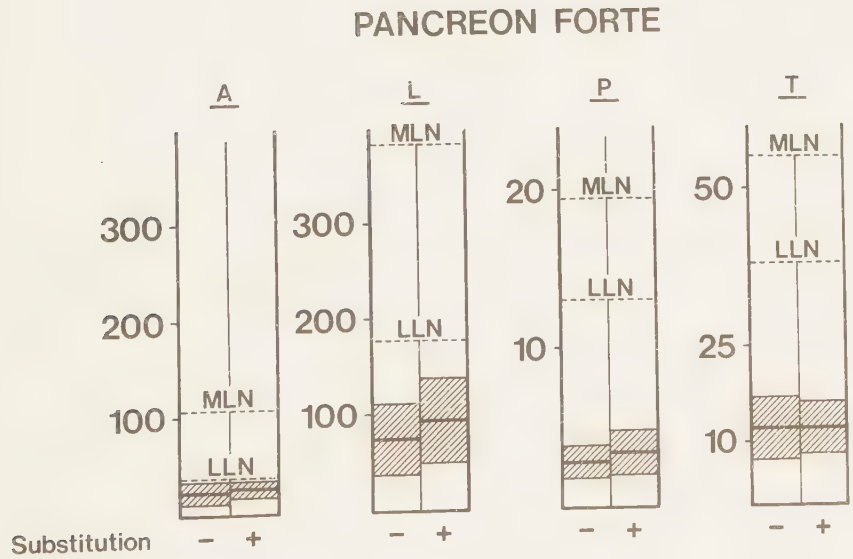


Fig. 2 A-B Intestinal enzymic activity in pancreatic insufficiency (24 patients) without (-) and with (+) pancreatin supplementation. Activities expressed according to Fig. 1. The values are given as mean ± S.E.M. * = $p<0.05$. ** = $p<0.01$. *** = $p<0.001$. MLN = Mean level of normals. LLN = Lower level of normals (reference 14). A = amylase, L = lipase, P = phospholipase and T = trypsin.

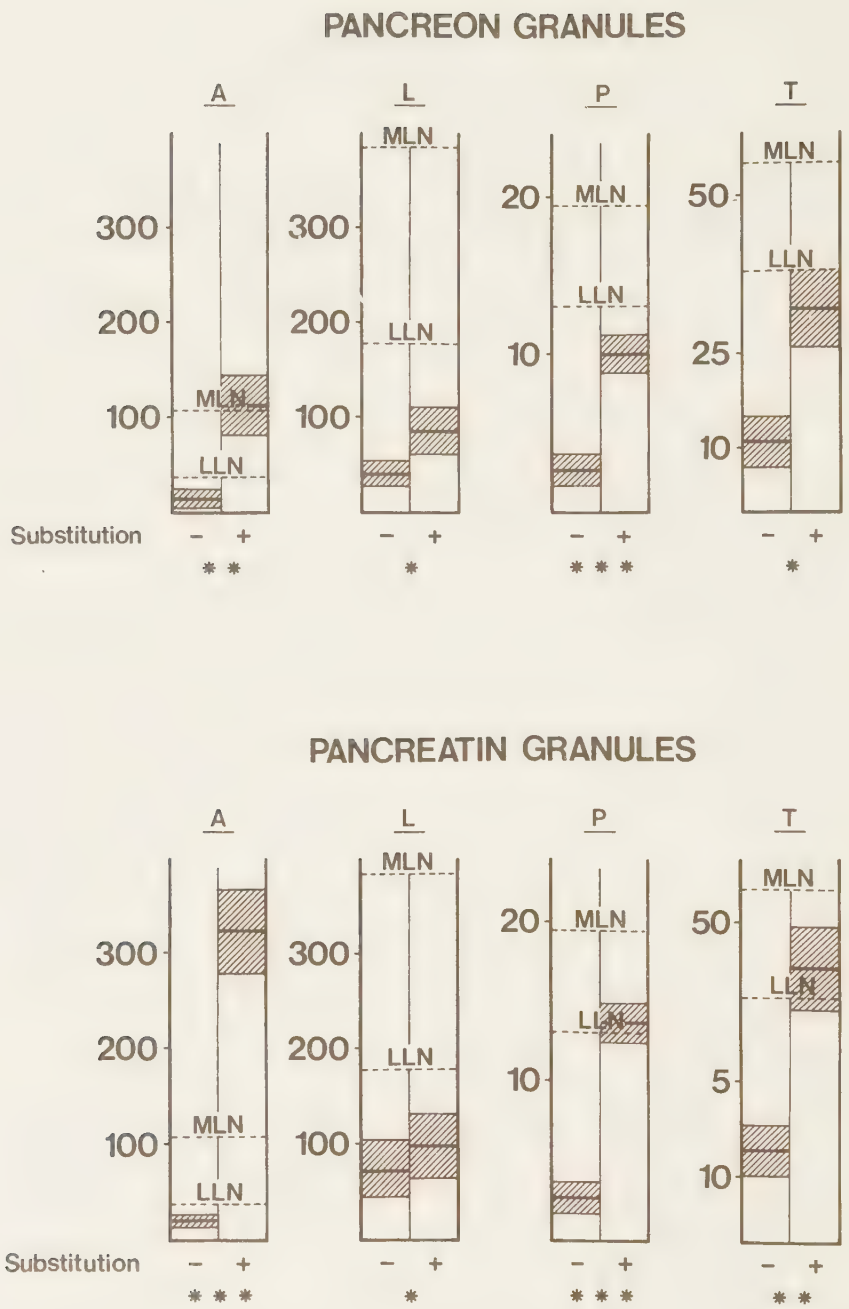


Fig. 2 C-D Intestinal enzymic activity in pancreatic insufficiency (24 patients) without (-) and with (+) pancreatin supplementation. Activities expressed according to Fig. 1. The values are given as mean ± S.E.M. * = $p<0.05$. ** = $p<0.01$. *** $p<0.001$. MLN = Mean level of normals. LLN = Lower level of normals (reference 14). A = amylase, L = lipase, P = phospholipase and T = trypsin.

Relations between in vitro and in vivo activities

Human small intestinal juice was used as incubation medium in the in vitro studies. We therefore found it legitimate to compare the enzyme concentrations of the administered test meal after addition of the different pancreatic extract and the increase in enzyme activity caused by the different preparations within small intestinal aspirates collected during two hours after the administration of the test meal. As seen in table II relatively higher activities of the enzymes in granulated form reached the small intestine as compared to those of the tablets especially regarding the amylase, phospholipase and trypsin activities.

Table II. Relations between the change of concentrations of pancreatic enzymes in intestinal aspirates after replacement therapy and the concentrations of pancreatic enzymes in the test meal given.

$$\left(\frac{\text{Change in enzyme activity/ml intestinal aspirate}}{\text{Enzyme activity/ml test meal}} \times 1000 \right)$$

	Amylase	Lipase	Phospholipase	Trypsin
Pancreozym (T)	82	130	189	73
Pancreon forte (T)	6	127	75	16
Pancreatin (G) granules	435	216	306	480
Pancreon (G) granules	209	370	230	297

DISCUSSION

Substitution therapy with pancreatic extracts has been employed in the treatment of pancreatic insufficiency for many years. However, there still remains a difference of opinion as to their value (17,23). Some authors have reported increased fat absorption as measured by a variety of parameters when enzyme therapy was instituted in pancreatic insufficiency (1,5,10,21). On the other hand Selle (25) found that fat digestion was not improved either by enteric-coated or non-coated pancreatin. Coffey et al. (3) using pancreatectomized dogs reported that faecal fat loss was unaffected by the addition of 2 g pancreatin daily to the animals' meals. Graveson (11) and Priestly et al. (19) studied patients after total pancreatectomy and observed no significant increase in fat absorption with enzyme therapy. In vitro analyses have demonstrated a considerable variation in enzyme content, especially of lipase, of the commercially available pancreatic supplements (7,9,18). Comparison

between reports on the in vitro activities of pancreatin preparations is, however, difficult due to different enzyme assays and units used.

In the present study, in which human intestinal juice was chosen as the incubation medium, high but varying enzyme activities of the four preparations were found (Fig. 1, Table I). The lipase activity per gram pancreatin was found to be highest in the preparation with the less complex manufacturing. This is in accordance with the suggestion that lipase is the most vulnerable of the pancreatic enzymes (24). When comparing the enzyme activities of the preparations from two different batches we found significant differences which is in accordance with previously reported experiences (7,18).

Indirect stimulation of the pancreas by means of a test meal as described by Borgström (2) has been found to be a valuable test of pancreatic function and digestive capacity in man (14,16). We found it suitable for an evaluation of the ability of pancreatic replacement preparations to affect the enzyme concentrations in the upper part of the small intestine. In the present study four supplements with different galenic properties were studied. In patients with pancreatic insufficiency the two preparations in tablet form - either protected against acid or not - did not cause any significant increase of enzyme concentrations in pooled intestinal aspirates during the first two hours after a test meal. Similarly the preparation manufactured as a simple tablet (Pancreozym^{R}) did not markedly influence the intestinal enzyme activities of healthy students. On the other hand the preparations in granulated form in patients with pancreatic insufficiency induced a statistically significant increase of the intestinal amylase, lipase, phospholipase and trypsin concentrations. These increased enzyme concentrations found in intestinal content after administration of preparations in granulated form might be partly explained by the difference in the doses given, at least regarding amylase and phospholipase. Comparison between pancreatic enzyme concentrations in the test meal given and in the intestinal aspirates (Table II), however, suggests that relatively greater amounts of active enzymes administered as granules reach the duodenum. This points to the importance of the galenic properties of the preparations. Supplements in granulated form may be more efficient possibly due to an adequate mixing with the food in the stomach and a continuous emptying to the intestine resulting in high levels of active enzyme within the duodenum at the time when food is passing into the small bowel.

The preparations which increased enzyme concentrations in the small intestine had special acid-protecting properties such as enteric coating or tannin-binding. On the other hand, one of the tablets which did not affect the intestinal enzyme concentrations also had acid protecting property. At present the value of antacids and enteric coating in combination with pancreatic replacement therapy is questioned (20). It has, however, recently been reported that H_2-receptor blockers markedly improve the ability of pancreatic supplements to augment the enzyme concentrations in the duodenum (20,22).

It has been shown that absorption of nutrients chiefly takes place in the very first part of the duodenum-jejunum (2). Therefore it seems logical to assume that supplements to give full effect should cause an increase of enzymic activity in the part of the intestine investigated in the study. Although we could not find any increase of lipase, after administration of the preparations in tablet form in the present study, reduction - but no normalization - of faecal fat excretion was registered (unpublished). The enzyme activities reported in this paper are pool values of 8 samples collected during 2 hours. Possibly the lipase concentrations actually were increased in some of these samples during the collection period which might explain the decreased faecal fat excretion. It has been shown by diMagno et al. (5) that although the peak values of lipase after supplementation were not more than 1% of the mean values of normals, a reduction of steatorrhea was obvious. Thus even minimal changes of intestinal enzyme concentration will influence the degree of steatorrhea in advanced pancreatic insufficiency. Our results are, however, in contrast to those of diMagno et al. (5) in so far that we found a relatively higher increase of the intestinal concentrations of pancreatic enzymes after granulated supplements.

In conclusion the results of the present study re-emphasize that the enzyme content of different commercial pancreatic enzyme preparations vary widely. Further there seemed to be a relation between the enzymic potency of the dose given and the increase of pancreatic enzyme concentrations in the small intestine after supplementation. This relationship is, however, probably only at hand when pancreatin is administered in granulated form. Thus, preparations in granulated form seem to be preferable to tablets due to their ability to increase the duodenal concentration of pancreatic enzymes.

ACKNOWLEDGEMENT

This work was supported by the Swedish Medical Research Council (Project No 17X-03012), the Medical Faculty of Lund and Albert Påhlsson Foundation.

REFERENCES

1. Berkowitz, D. & Shapiro, B.
Am. J. Gastroent. 1964, 41, 45-50

2. Borgström, B., Dahlquist, A., Lundh, G. & Sjövall, J.
J. Clin. Invest. 1957, 36, 1521-1536

3. Coffey, R.J., Mann, F.C. & Bollman, J.L.
Am. J. Dig. Dis. 1940, 7, 149-154

4. Dahlqvist, A.
Scand. J. Clin. Lab. Invest. 1962, 14, 145-151

5. Di Magno, E.P., Malagelada, J.R., Go, V.L.W. & Moertel, C.G.
N. Engl. J. Med. 1977, 296, 1318-1322

6. Di Magno, E.P, Go, V.L.W. & Summerskill, W.H.J.
N. Engl. J. Med. 1973, 288, 813-815

7. Drummond, S., Saunders, J.H.B., Leach, R. & Wormsley, K.G.
Scott. Med. J. 1977, 22, 221-224

8. Erlanson, C. & Borgström, B.
Scand. J. Gastroent. 1970, 5, 293-295

9. Guilian, B.B., Singh, L.M., Mansfield, A.O., Pairent, F.W. & Howard, J.H.
Ann. Surg. 1967, 165, 564-570

10. Graham, O.Y.
N. Engl. J. Med. 1977, 296, 1314-1317

11. Graveson, K.J.
Am. J. Dig. Dis. 1962, 7, 473-482

12. Hummel, B.C.W.
Canad. J. Biochem. Biophys, 1959, 37, 1393-1399

13. Ihse, I. & Arnesjö, B.
Acta Chem. Scand. 1973, 27, 2749-2756

14. Ihse, I., Arnesjö, B., Kugelberg, C. & Lilja, P.
Scand. J. Gastroent. 1977, 12, 663-668

15. Lundh, G.
XXI Congrès de la Société Internationale de Chirurgie, Philadelphia, 1965

16. Lundh, G.
Gastroenterology, 1962, 42, 275-280

17. Meyer, J.H.
N. Engl. J. Med. 1977, 296, 1347-1348

18. Pairent, F.W. & Howard, J.M.
Arch. Surg. 1975, 110, 739-741

19. Priestly, J.T., Comfort, M.W. & Radcliffe, J. Jr.
Ann. Surg. 1944, 119, 211-216

20. Regan, P.T., Malagelada, J.R., Di Magno, E.P., Glanzman, S.L. & Go, V.L.W.
N. Engl. J. Med. 1977, 297, 854-858

21. Ross, C.A.C.
Arch. Dis. Child. 1955, 30, 316-321

22. Saunders, J.H.B., Drummond, S. & Wormsley, K.G.
Brit. Med. J. 1977, 1, 418-419

23. Saunders, J.H.B.
Gut 1975, 16, 157-162

24. Schön, H.
Symposium of the 2nd World Congress on Gastroenterology, Munich, 1962

25. Selle, W.A.
J. Nutr. 1937, 13, 15-21

Accepted for publication in Scandinavian Journal of Gastroenterology

The present thesis is based on the following papers:

I Trypsin as a regulator of pancreatic enzyme secretion in the rat. (In collaboration with I. Ihse and I. Lundquist.) Scand. J. Gastroent. In press.

II Effects of amylase, lipase, trypsin, and bile on pancreatic enzyme secretion in the rat. Submitted for publication.

III Vagal influences on the pancreatic response to intraluminal trypsin. (In collaboration with I. Ihse and I. Lundquist.) Submitted for publication.

IV Effects of intestinal amylase and trypsin on pancreatic secretion in the pig. (In collaboration with I. Ihse.) Scand. J. Gastroent. In press.

V Feedback regulation of pancreatic enzyme secretion by intestinal trypsin in man. (In collaboration with I. Ihse and I. Lundquist.) Digestion 1977, 15, 303-308.

VI Intestinal concentrations of pancreatic enzymes following pancreatic replacement therapy. (In collaboration with I. Ihse and I. Lundquist.) Scand. J. Gastroent. In press.

The above articles will be referred to in the text by their respective Roman numerals.

CONTENTS

A. Physiology of pancreatic secretion - current concepts..... 7

B. Relationships between intraduodenal bile-pancreatic juice components and pancreatic secretion; previous investigations..... 8

C. Purpose and plan of the present investigation..... 9

D. Material.
D1. Rats, pigs, patients and healthy volunteers..... 10
D2. Chemicals and drugs..... 11

E. Methods.
E1. Experiments in rat..... 13
E2. Experiments in pig..... 14
E3. Experiments in man..... 15
E4. In vitro studies..... 16
E5. Biochemical assays..... 16
E6. Calculations..... 16

F. Influences on pancreatic enzyme secretion of deviation and re-infusion of (bile-)pancreatic juice..... 17

G. Influences on pancreatic enzyme secretion of intraduodenal amylase, lipase,and trypsin..... 21

H. Influence on pancreatic enzyme secretion of intraduodenal bile..... 24

I. Studies on possible mechanisms of trypsin-induced effects on pancreatic enzyme secretion..... 26

J. Intestinal pancreatic enzyme activities following enzyme substitution therapy..... 29

K. Feedback regulation of pancreatic enzyme secretion through variations of intraluminal trypsin; possible clinical implications..... 33

L. Summary..... 34

M. Acknowledgements..... 35

N. References..... 36

A. PHYSIOLOGY OF PANCREATIC SECRETION - CURRENT CONCEPTS

For many years the autonomic nervous system was believed to be the only regulator of the pancreatic secretion. However, after the discovery of gastrointestinal hormones this concept was modified, and it is now evident that there is a close inter-relationship between neural and hormonal mechanisms in the regulation of exocrine pancreatic secretion (For references see 6, 33, 61, 76).

The mechanisms behind and the functional importance of the neural control of pancreatic secretion are not fully understood. It is known that electrical stimulation of vagal fibers as well as indirect vagal stimulation by e.g. insulin or 2-deoxyglucose results in an increased pancreatic enzyme secretion. After truncal vagotomy the pancreatic fluid, bicarbonate, and enzyme secretion stimulated by food is markedly reduced, while exogenous hormonal stimulation provokes a normal pancreatic secretory response. Administration of acetylcholine enhances pancreatic secretion, and atropine causes a marked and prolonged decrease in pancreatic flow rate and protein and bicarbonate output after endogenous release of cholecystokinin-pancreozymin (CCK-PZ). Also by more direct ways it has been shown that vagal reflexes influence on pancreatic exocrine secretion. Stimulation of the central cut end of one vagus nerve stimulates pancreatic secretion via the contralateral intact vagus. Again, distension of the stomach is found to activate the vago-vagal reflex to the pancreas. The effect of sympathetic stimulation seems to be complex, as both excitatory and inhibitory effects on pancreatic enzyme secretion are attributed to splanchnic nerve stimulation. Furthermore, sympathetic nerves may act indirectly by circulatory changes or by a combination of both circulatory and direct effects.

Acidification of the duodenum provokes pancreatic secretion by the release of secretin from the intestinal mucosa. Secretin has been demonstrated mainly to stimulate pancreatic secretion of fluid and bicarbonate. Certain components of food, especially fat and protein, are capable of stimulating pancreatic enzyme secretion when present within the duodenal lumen. This observation led to the discovery of CCK-PZ, a hormone produced in the wall of the proximal small intestine. In addition to its strong action as a stimulant of enzyme secretion CCK-PZ is a weak stimulant of bicarbonate secretion when acting alone, but markedly augments bicarbonate secretion stimulated by secretin. Similarly, secretin, in addition to its strong action as a stimulant of bicarbonate secretion, is a weak stimulant of enzyme secretion when acting alone,and augments the enzyme secretion stimulated by CCK-PZ.

Additional polypeptides may be involved in the control of pancreatic secretion. Glucagon has been shown to inhibit pancreatic secretion,but the precise role of pancreatic glucagon and enteroglucagon in the physiological control of pancreatic secretion is not established. Vasoactive intestinal polypeptide (VIP) is reported to be a weak stimulant of pancreatic secretion. Gastrin stimulates pancreatic flow rate and protein

output in rat, rabbit and dog. In man, however, it is questionable if gastrin has got any effects on pancreatic secretion. Chymodenin evokes a prompt secretion of pancreatic juice with a high concentration of chymotrypsinogen, but only a moderate change in the release of other pancreatic enzymes. Somatostatin has been shown to inhibit exocrine pancreatic secretion. Lately, also other polypeptides which may influence pancreatic secretion, have been found, such as pancreatic polypeptide (PP), bombesin, motilin and cerulein.

The response of exocrine pancreatic secretion to ingestion of food is generally divided into three phases: the cephalic, the gastric and the intestinal phase. The cephalic phase seems to be mediated via vagal release of gastrin from the gastric antrum in the dog, while in man it is not known whether the cephalic phase in pancreatic secretion is mediated directly by neural impulses to the pancreas or indirectly through the vagal release of gastrin and/or CCK-PZ. During this phase, the pancreas mainly secretes proteins. The gastric phase is initiated, when food enters the stomach. Gastrin is released by mechanical and chemical stimuli. In fact, the existence of a gastric phase of pancreatic secretion in man may be questioned, as it has not been convincingly shown that gastrin provokes pancreatic secretion in man. During the gastric phase it is generally accepted, that it is mainly the enzyme secretion which is stimulated. When food passes into the duodenum the intestinal phase starts. Under the influence of digestive products and hydrochloric acid on the duodenal mucosa, CCK-PZ and secretin are released, resulting in pancreatic secretion of proteins, fluid and bicarbonate. Furthermore, distension of the duodenum by food provokes pancreatic secretion.

An essential factor regulating the termination of the pancreatic secretory response seems to be the rapid turnover of the hormones. Furthermore, two inhibitory mechanisms of pancreatic secretion have been suggested. One seems to operate in the duodenum, where the release from the endocrine cells of stimulatory factors is thought to be decreased by intraluminal proteases. Another suggested mechanism is exerted by an inhibitory peptide (pancreotone), which has been reported to be released from the ileal and colonic mucosa by digested foodstuffs, especially fatty acids. In addition, as mentioned above, certain hormones also inhibit protein secretion by acting directly on the acinar cells (glucagon, somatostatin).

B. RELATIONSHIPS BETWEEN INTRADUODENAL BILE-PANCREATIC JUICE COMPONENTS AND PANCREATIC SECRETION; PREVIOUS INVESTIGATIONS

Investigations during the past years suggest a relationship between intraduodenal tryptic activity and pancreatic morphology and function. Long-term administration of raw soy beans or trypsin inhibitor to chickens or rats results in pancreatic hypertrophy (2, 9, 40). Furthermore, the enlarged pancreatic glands have got an increased capacity to secrete digestive enzymes (36, 55). A single feeding of a meal contai-

ning raw soy beans or trypsin inhibitor induces an immediate secretory response of the pancreas (57). In these studies, enzyme secretion has generally been determined indirectly by measuring the changes of enzyme activities within the pancreas and/or within the small intestinal contents.

It was not until 1951 that Annis and Hallenbeck (1) in a more direct way demonstrated a relation between intraduodenal pancreatic juice and pancreatic secretion. After experiments performed on dogs they reported that if pancreatic juice was permanently removed from the duodenum, the total volume of juice secreted was significantly greater than was the case when the juice was replaced into the duodenum. Their findings were confirmed by Cook et al. (10), reporting that exclusion of pancreatic juice from the duodenum resulted in an increase in the bicarbonate output and the flow rate from the dog pancreas.

In 1958, Grossman (30) reported pancreatic hypersecretion in rats with external pancreatic fistulae. Later Green and Lyman (28) by direct methods suggested that pancreatic enzyme secretion in the rat was subjected to a feedback regulation by intestinal trypsin. The mechanism by which trypsin or pancreatic juice may suppress the pancreatic enzyme secretion is not fully elucidated. It is suggested that trypsin may interfere with the release of a humoral factor, or factors, from the intestine stimulating pancreatic secretion. The possible role of neural pathways in this context is not known.

C. PURPOSE AND PLAN OF THE PRESENT INVESTIGATION

The present study constitutes a link in a series of investigations (36-44) the aim of which is to examine the proposed feedback regulation of exocrine pancreatic secretion and its possible clinical and therapeutic implications in pancreatic disease. Hitherto intraluminal trypsin and chymotrypsin (28) have been claimed to play a regulatory role. Whether other components of bile-pancreatic secretion also are involved is not known.

One purpose of the investigation was to elucidate further the influence on rat pancreatic secretion of intraluminal bile-pancreatic juice and trypsin, respectively, with special reference to the secretory pattern of different pancreatic enzymes. Comparative studies were to be performed in pig and man. Another purpose was to investigate whether intraduodenal amylase, lipase, and pure bile exerted any feedback effects on rat pancreatic enzyme secretion.

The results of previous investigations are consistent with a hormonal mediation of the observed feedback regulation (73). Studies on the nervous control mechanisms are, however, only sparsely reported. Therefore, the effects of vagotomy, cholinergic blockade and vagal stimulation by insulin-induced hypoglycaemia on the pancreatic response to intraduodenal trypsin were studied in the rat.

The ability of pancreatic extracts to influence the activities of digestive enzymes within the intestinal lumen in patients was until recently unknown. If a feedback regulation of pancreatic secretion exists also in man, it was considered important to investigate whether ingested pancreatic extracts reach the duodenum in amounts sufficient to interfere with such a regulation. Therefore a study on digestive enzyme activities following enzyme substitution therapy was performed in healthy students as well as in a series of patients suffering from exocrine pancreatic insufficiency.

D. MATERIAL

D1. Rats, pigs, patients and healthy volunteers

Experiments in rat

Male Wistar rats weighing 250 - 300 g were used. The animals had free access to tap water and were kept on a standard pellet diet which was withdrawn immediately before the surgical procedures. Postoperatively, the rats were kept in restraining cages and had access to a fluid composed of one part Ringer solution and one part 5.5 % glucose solution, which was withdrawn before the start of the experiments.

Experiments in pig

Male pigs weighing 15-17 kg were used. Food and water were withdrawn at least six hours before surgery. Postoperatively, the pigs were kept in restraining cages, where they had free access to water and were given solid food three times daily. The animals were fasted 8 hours before the start of the experiments.

Experiments in man

A series of experiments was performed on a 70-year-old man, in whom a tumour of the papilla Vateri completely obstructed the bile-pancreatic flow into the intestine as demonstrated by PTC (Percutaneous Transhepatic Cholangiography). It was found that the pancreatic duct communicated with the bile duct via a common channel (Fig. 1).

Nine healthy students and 24 patients participated voluntarily in the pancreatic replacement study. The patients suffered from exocrine pancreatic insufficiency proven by the meal provocation test described by Lundh (53). In 10 of the patients, the diagnosis was chronic pancreatitis and in 8 non-operated pancreatic cancer. In 6 other patients a total pancreatectomy had been performed prior to the investigation. All subjects gave their informed consent to the study, the plan of which was accepted by the Ethical Committee of the University of Lund.

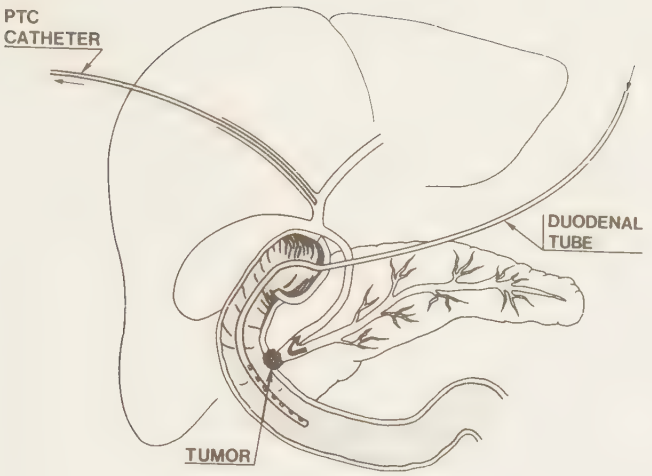


Fig. 1 Diagram showing the localization of the tumour and the catheters in a patient subjected to experiments. Bile-pancreatic juice was sampled from the PTC catheter. Substances were infused via the duodenal tube. (Reprinted from paper V.)

D2. Chemicals and drugs

Experiments in rat

Highly purified crystalline amylase (type II-A from Bacillus Subtilis; 4 x crystallized; Sigma Ltd, St. Louis, USA) was dissolved in 0.05 M $NaHCO_3$. The activity of the solution was 100 000 μkat/l as determined by the Phadebas® method (AB Pharmacia, Uppsala, Sweden)(8). Porcine pancreatic lipase purified according to the method described by Donnér (19) was a gift from Dr Charlotte Erlanson, University of Lund, Sweden. The lipase was dissolved in 0.05 M $NaHCO_3$ in an amount giving an activity of 250 - 300 U/ml as analysed with tributyrine as the substrate (21). Porcine crystalline trypsin (A/S Novo, Copenhagen, Denmark) was dissolved in 0.05 M $NaHCO_3$ corresponding to a tryptic activity of 350 U/ml (2.5 mg/ml), 700 U/ml (5 mg/ml) and 1 400 U/ml (10 mg/ml) as analysed with TAME (þ-toluene-sulphonyl-L-arginine methyl ester) as the substrate (34). Highly purified bovine lung trypsin inhibitor was obtained from Bayer A/G, Leverkusen, West Germany. This inhibitor is not absorbed from the intestine (82) nor digested by intestinal proteolytic enzymes (24). It inactivates trypsin, chymotrypsin, plasmin and kallikrein, but does not affect elastase or subtilisin (24). The inhibition is competitive and complexes are formed at a pH optimum of 8.0 (24). In ancillary experiments it was found that 0.5 mg of the inhibitor completely abolished the tryptic acticity of 1 mg of the trypsin used. Lyophilized enterokinase was obtained from Miles Laboratories Inc, Elkhart, USA. The anticholinergic drug propantheline (2-hydroxy-ethyl) diisopropyl-methyl-ammonium

bromide xanthene-9-carboxylase (Pro-Banthine®, G D Searle & Co, Chicago, USA) and Insulin Vitrum® (AB Vitrum, Stockholm, Sweden) were used in some experiments.

Bile or (bile-)pancreatic secretion used for infusion was collected on ice from animals provided with bile duct or (bile-)pancreatic duct fistulae, respectively, and frozen at -20°C the day before the experiments. The bile collected was found to be free from tryptic (trypsinogen) activity.

Experiments in pig

Porcine crystalline trypsin was obtained from A/S Novo, Copenhagen, Denmark. It was dissolved in 0.05 M $NaHCO_3$ at a concentration of 1 mg/ml. The tryptic activity of this solution was 140 U/ml assayed with the use of TAME (þ-toluene-sulphonyl-L-arginine methyl ester) as the substrate (34). This activity roughly corresponded to the tryptic activity of the pancreatic juice of the pig. Crystalline porcine amylase was obtained from Sigma Ltd, St. Louis, USA. It was dissolved in 0.05 M $NaHCO_3$ at a concentration of 50 mg/ml. The amylolytic activity of this solution was 500 U/ml assayed with maltose as the substrate (14). This activity roughly corresponded to the amylolytic activity of the pancreatic juice of the pig. In ancillary experiments the amylase preparation was found to be slightly polluted with trypsin(ogen). Highly purified bovine lung trypsin inhibitor was obtained from Bayer A/G, Leverkusen, Germany. Pancreatic juice for intraduodenal infusion was collected on ice the day before the experiment and frozen at -20°C.

Experiments in man

Porcine crystalline trypsin (A/S Novo, Denmark, Copenhagen) was dissolved in 0.05 M $NaHCO_3$ at a concentration of 1 mg/ml. Bile-pancreatic juice was collected on ice from the percutaneous transhepatic catheter draining the bile and pancreatic ducts (PTC catheter).

Four commercially available preparations of pancreatin with different galenic and adjunctive protective properties against acid were used. One preparation was a simple tablet without protective devices (Pancreozym®, AB Tika, Sweden), one a tablet with tannin-bound enzymes, an arrangement which by the manufacturer is claimed to function as protection against low pH and pepsin (Pancreon forte®, AG Kali-Chemie, West Germany), one an enteric-coated granulated preparation (Pancreatin granules®, A/S Rosco, Denmark), and one a granulated preparation with tannin-binding (Pancreon granules®, AG Kali-Chemie, West Germany).

Human intestinal juice used as incubation medium in the in vitro studies of pancreatin preparations was collected on ice from healthy subjects after ingestion of a test meal as described by Lundh (53). The same type of test meal was used in the human in vivo experiments.

E. METHODS

E1. Experiments in rat

The rats were operated upon under light ether anesthesia with clean but not sterile instruments. A midline incision was made exposing the duodenum and the bile-pancreatic duct. A polyethylene catheter with an inner diameter of 0.58 mm and a length of 15 cm (Intramedic PE 50) was introduced into the bile-pancreatic duct via an incision close to the intestinal wall. Another catheter was introduced into the duodenum 5 mm below the pylorus to permit direct infusion of substances into the intestine. Separate skin incisions were used to bring out the catheters. At the end of the operation 10 - 15 cc saline was injected subcutaneously. No antibiotics were administered. Postoperatively the rats were maintained in restraining cages and allowed 13 - 14 hours of surgical recovery. When using this schedule the postoperative mortality was less than 5 %.

Some rats were subjected to the same operation as described above, but in addition a truncal vagotomy was performed by dividing the right and left vagus nerves just below the diaphragm according to the technique described by Shay et al. (75). After the experiments the completeness of the vagotomy was checked according to the method described by Håkanson et al. (35). In all rats used the vagotomy was found to be complete.

All experiments were performed 15 hours after the surgical procedure, and only one experiment was performed on each rat. Animals in which complications occurred, such as catheter dislodgement or bleeding, were excluded from the study. The intestinal infusions were administered through the duodenal fistula using a pump giving a constant infusion rate of 1.2 ml/h, which roughly corresponded to the volumes spontaneously secreted via the bile-pancreatic duct fistulae. The pH of the infusates was corrected to 8.0 $\pm$ 0.2. The solutions for infusion were kept on ice, and the temperature brought to +25°C immediately, before the introduction into the duodenum (Fig. 2). Bile-pancreatic secretion was collected in small pre-weighed tubes on ice at 30 minutes' intervals. The samples were immediately frozen at -20°C and analysed in duplicate within two weeks.

In the present series of experiments combined bile-pancreatic secretions were collected instead of pure pancreatic juice, in order to avoid problems with the very low flow rate when the latter juice is collected from the rat. Pancreatic juice is a viscous substance, and bile may normally assist the passage of it down the common bile duct. Any errors in estimating pancreatic secretory rate brought about by diverting bile from the common bile duct were therefore avoided by the collection of combined bile-pancreatic juice.

Pancreatic enzyme activities were estimated by measuring the output of individual pancreatic enzymes (amylase, lipase and/or trypsinogen). Furthermore protein output was assayed and found to be secreted in a parallel fashion with the enzymes. In some experiments therefore only protein output was determined and considered closely to reflect pancreatic enzyme secretion.

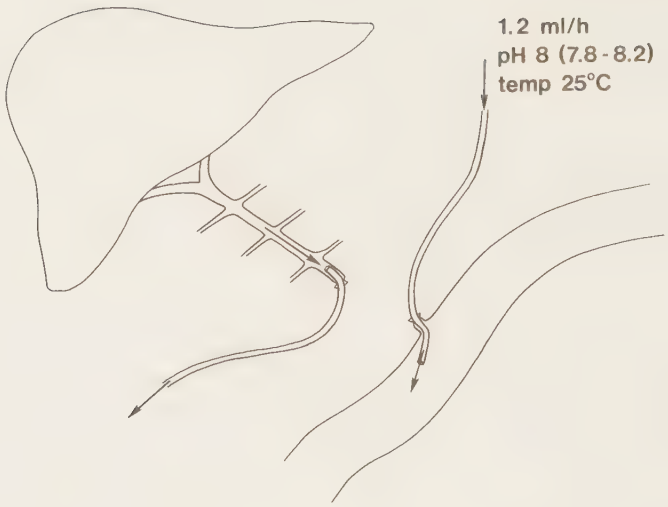


Fig. 2 The preparation in rat. Cannulae were inserted into the bile-pancreatic duct and the duodenum, respectively. Rate, pH and temperature of the infusates were kept constant during administration.

E2. Experiments in pig

The pigs were intubated orally and anesthetized with halothane (Fluothane®), N_2O and O_2. Sterile instruments were used throughout the operations. A midline abdominal incision exposed the upper viscera. The pancreatic duct was divided at the point of its entry into the intestine and cannulated with a baby feeding tube,which had an outer diameter of 3.5 mm. A duodenal fistula was established by inserting a polyethylene tube through an incision in the intestinal wall 10 cm distal to the pylorus (Fig. 3). The two catheters were brought out through separate abdominal wall incisions. The animals were allowed 48 hours of surgical recovery before the experiments were started. During this period the pancreatic juice was returned into the intestine by joining the pancreatic duct cannula and the duodenal cannula. No antibiotics were used,and no postoperative deaths occurred. The experiments were performed in restraining cages after 8 hours of fasting. During this period and during the experiments an infusion of isotonic 5.5 % glucose solution with the addition of 80 mmol/l NaCl was administered intravenously at a rate of 50 ml/hour. Duodenal infusates were administered via a pump at a constant infusion rate of 1.5 ml/minute,which corresponded to double the volume secreted from the pancreas. The pH of the infusates was corrected to 8.0 ± 0.2,and the temperature was brought to +25°C immediately before the infusion into the duodenum (Fig. 3). Secretions were collected from the pancreatic fistulae in pre-weighed tubes on ice at 90 minutes' intervals and immediately frozen at -20°C until analyzed in duplicate within two weeks.

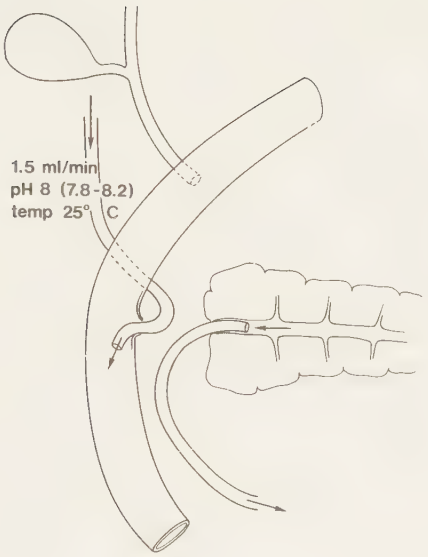


Fig. 3 The preparation in pig. Cannulae were inserted into the pancreatic duct and the duodenum, respectively. Rate, pH and temperature of the infusates were kept constant during administration. (Reprinted from paper IV.)

E3. Experiments in man

In a patient with a tumour of the papilla Vateri, which completely obstructed the pancreatic flow and directed it into the bile duct via a common channel, the pancreatic secretion could be collected via a PTC catheter reaching the bile duct system (Fig. 1). A duodenal tube was inserted with the tip a few cm distal to the papilla (Fig. 1). This arrangement allowed substances to be infused into the duodenum and the bile-pancreatic secretion to be collected. Substances were infused at a constant rate of 1 mg/min, and the bile-pancreatic juice was collected on ice in 15 minutes' periods in pre-weighed tubes.

In the experiments concerning pancreatic substitution therapy the patients and the healthy subjects were investigated after 12 hours' fasting without premedication. A duodenal tube (Levine n:o 12) was inserted under fluoroscopic control with its tip at the ligament of Treitz. In patients who had undergone total pancreatectomy, the tip of the tube was positioned 40 cm distal to the gastrojejunostomy. The 24 patients were randomly allocated into four groups with six in each. A test meal according to Lundh, either without or with a pancreatin preparation, was given to each subject on two different days in random order. Twice the recommended dosage (eight tablets of Pancreozym®, four tablets of Pancreon forte®, 20 ml of Pancreatin granules® or Pancreon granules®), was administered together with the test meal. The sampling was started immediately after the administration of the meal. During the first hour the duodenal aspirates were sampled in 10 minutes' fractions and during

the second hour in 30 minutes' fractions. From each sample one ml was withdrawn after shaking for 1 - 2 minutes and pooled. All collections were made on ice and stored at -20°C until analysed. Enzyme determinations were performed in duplicate.

E4. In vitro studies

One pancreatin tablet or 10 ml of the pancreatin preparations in granulated form was crushed in a mortar and incubated at a temperature of +37°C with 50 ml pooled human duodenal juice obtained from normal subjects. The estimated enzyme activities of pancreatin were found to be independent of the duration of incubation at the periods of time studied (10, 25, 40 and 55 minutes, respectively). In the present study an incubation time of 40 minutes was chosen. Six tablets or six ml dosages from two separate batches of each pancreatin preparation were analysed. The activities of amylase, lipase, phosholipase and trypsin of the incubation medium (human duodenal juice) were estimated before and after incubation with a preparation. Thus, the enzyme activities of the different preparations were calculated as the activities estimated after incubation minus those estimated before addition of the pancreatin preparation to the incubation medium.

E5. Biochemical assays

Protein was determined according to Lowry et al. (52) as modified by Eggstein and Creutz (20). Lipase was assayed by a scaled-down version of the method described by Erlanson and Borgström using tributyrine as the substrate (21). Amylase was determined either according to Dahlqvist (14) or by the Phadebas® method (8) commercially available by AB Pharmacia, Uppsala, Sweden. Trypsin was estimated by a modified Hummel method using TAME (p-toluene-sulphonyl-L-arginine methyl ester) as the substrate (34). Phospholipase was determined according to Ihse and Arnesjö (37). Trypsinogen was estimated by activation of zymogens in bile-pancreatic juice by enterokinase (Miles Laboratories Inc., Elkhart, USA). A mixture of 100 µl diluted bile-pancreatic juice (1:10), 200 ml 0.05 M Tris-HCl buffer, pH 8.0, containing 0.02 M $CaCl_2$ and 200 µl enterokinase (0.05 mg/ml in re-distilled water) was incubated at +25°C for two hours. The reaction was stopped by addition of 10 µl 2 M HCl. The tryptic activity was determined by the modified Hummel method mentioned above.

E6. Calculations

The data obtained were subjected to statistical analysis using the Student's *t*-test for paired and unpaired observations, respectively. All values are given as the mean ± SEM.

F. INFLUENCE ON PANCREATIC ENZYME SECRETION OF DEVIATION AND REINFUSION OF (BILE-)PANCREATIC JUICE

The results of the present investigation showed that exclusion of (bile-) pancreatic juice from the intestine in rat, pig, and man caused an enhanced pancreatic enzyme secretion which was abolished by reinfusion of the (bile-)pancreatic juice. In pig and man alterations in secretory volume were more pronounced than in rat. The inhibitory effect of intraduodenal (bile-)pancreatic juice on pancreatic enzyme secretion was reversed after addition to the infusates of trypsin inhibitor in amounts sufficient to remove all tryptic activity. The output of amylase, lipase, and trypsinogen was parallel both during stimulation (exclusion of bile-pancreatic juice from the intestine or infusion of trypsin inhibitor in the presence of bile-pancreatic juice) and inhibition (re-infusion of bile-pancreatic juice) of pancreatic enzyme secretion.

In rats provided with bile-pancreatic fistulae the spontaneous secretion following cannulation of the bile-pancreatic duct was characterized by small fluctuations in secretory volume and by an unstable protein output during the first 13 to 14 hours, after which the protein output remained stable (Fig. 4) (I, II). Since in all other experiments the measurement of the pancreatic enzyme activities and of the protein contents of the mixed bile-pancreatic secretion gave parallel values (I, II) only the protein output was estimated in this experiment. Increased pancreatic enzyme secretion following diversion of (bile-)pancreatic juice has been reported by others (28, 30, 67). Green et al. (29) studied chymotrypsin output in rats for 8 hours after diversion of the bile-pancreatic juice and observed an immediate increase, which lasted for 3 hours and then a decrease to a new level of a higher basal rate of secretion. In the present experiments (I, II, III) the animals were given subcutaneous injections of fluid (10 ml 0.9 % NaCl every 10 hours) while in the study of Green et al. no fluid restitution was reported. This might possibly explain the decline in enzyme output found in their experiment.

Introduction of 0.05 M $NaHCO_3$ into the duodenum was without effect on the enzyme secretion and on the flow rate in rats with their bile-pancreatic juice deviated for more than 15 hours (Fig. 5) which clearly demonstrates the stable state of secretion under which the experiments were performed (I, II, III). Grossman (30) using a similar experimental model reported that the pancreatic secretion in fasting rats was uninfluenced by pyloric ligation and gastric drainage.

It is not fully elucidated whether the pancreas is maximally stimulated during the state of hypersecretion observed after deviation of (bile-) pancreatic juice. Petersen and Grossman (67) did not find any change in protein secretion after giving CCK-PZ to fistula rats suggesting that the pancreas was already maximally stimulated. On the other hand, Schneeman et al. (73) noted an increased secretory rate after exogenous

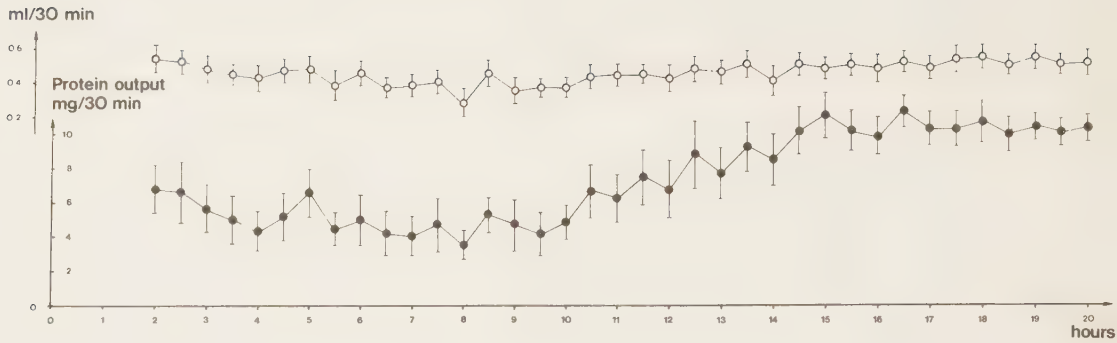


Fig. 4 Spontaneous secretory volume (○—○) and protein outpout (●—●) in conscious rats provided with bile-pancreatic fistulae (n = 5). Protein output was somewhat unstable during the first 13-14 hours after which it stabilized. Values are given as mean ± SEM.

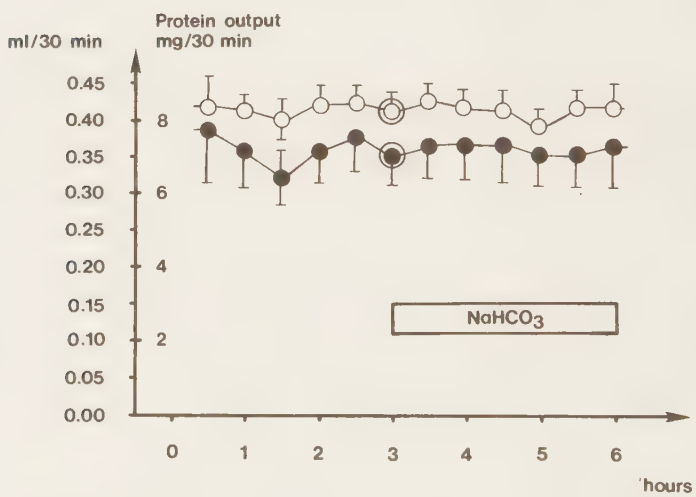


Fig. 5 Secretory volume (○—○) and protein output (●—●) in conscious rats (n = 7) provided with bile-pancreatic fistulae. Sampling started 15 hours after surgery. 1.2 ml/hour of 0.05 M $NaHCO_3$, pH 8.0 ± 0.2 was infused intraduodenally. The circles represent the preinfusion points. Values are given as mean ± SEM. (The results are taken from paper I.)

CCK-PZ in rats with chronic pancreatic fistulae. Grossman (30) in a similar experiment demonstrated a rise in pancreatic secretory volume after exogenous secretin. Furthermore, Evander et al. (22) found a pronounced increase of protein output in fistula rats after cerulein administration. These discrepancies are difficult to explain but may be due to methodological differences such as subcutaneous saline administration or not and duration of the diversion of the juice prior to the experiments.

Most studies on the feedback regulation of pancreatic secretion have dealt solely with the release of enzymes (I, II, III, 28, 29, 73). Recently, Petersen and Grossman (67) reported that bile-pancreatic juice depressed bicarbonate secretion as well. Basal bicarbonate output was depressed by 43 % and basal protein output by 59 %, when bile-pancreatic juice was reintroduced into the duodenum. This observation might have essential implications when discussing the mechanisms behind the observed feedback regulation (see chapter I).

Also in the pig deviation of pancreatic juice from the intestine resulted in pancreatic hypersecretion (IV). Similar observations were made by Corring (11) who in accordance with the present study also found that intestinal infusion of $NaHCO_3$ did not influence the pancreatic secretory volume nor the protein output in the pig (IV). In man (a patient with a tumour obstructing the ampulla of Vater) an excessive secretion of about 2 500 - 3 000 ml/24 h from the PTC catheter draining both the bile and pancreatic ducts (Fig. 1) was observed. This state of hypersecretion was uninfluenced by intraduodenal administration of 0.05 M $NaHCO_3$ (V). Thus, from the present findings in rat (I, II, III), pig (IV), and to some extent in man (V), and from reports by other authors (11, 28, 30, 67, 73) it seems as if exclusion of (bile-)pancreatic juice from the intestine results in a state of pancreatic hypersecretion which is uninfluenced by intraduodenal administration of $NaHCO_3$. The question whether the pancreas is maximally stimulated remains to be solved.

Re-introduction of bile-pancreatic juice into the duodenum in fistula rats inhibited protein output within one hour of re-introduction (I). This suppression remained for at least 3,5 hours as shown in figure 6 (I). Re-introduction of pancreatic juice in the pig depressed both the pancreatic secretory volume and the protein output significantly (IV). Also, in repeated experiments in the man with a PTC catheter draining both the pancreatic and bile ducts it was found that re-introduction of the patient's own bile-pancreatic juice into the duodenum depressed the secretory volume and amylase and lipase output significantly (V). Magee and Hong (59) suggested that the depression of pancreatic secretion observed after re-introduction of pancreatic juice in the pig was a result of increased intraduodenal pH. The findings of the present study do not support this hypothesis but rather point to other mechanisms in the regulation of pancreatic secretion. In man and in pig - in contrast to conditions in rat - the changes in the secretion were mainly a result of alterations in volume rather than in enzyme secretion (I, IV, V). If these findings reflect different mediators operating in the regulation of pancreatic secretion is not known.

The inhibitory effect on the pancreatic enzyme secretion induced by

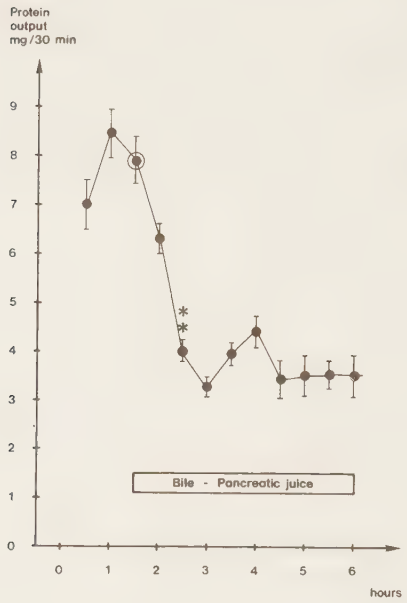


Fig. 6 Protein output in conscious rats (n = 11) provided with bile-pancreatic fistulae. 1.2 ml/hr of bile-pancreatic juice was reintroduced into the duodenum. The circle represents the preinfusion value used in the statistical calculation. Values are given as mean ± SEM. ** = $p < 0.01$ (Reprinted from paper I.)

intraduodenal (bile-)pancreatic juice was reversed in the species studied when trypsin inhibitor was added to the infusate in amounts sufficient to eliminate all intraduodenal tryptic activity (I, IV, V). These data support previous studies in the rat (28, 29). They suggest, however, that trypsin may play a key role in the feedback regulation of pancreatic enzyme secretion even in pig and man (see chapter G).

The Babkin hypothesis (3) of parallel secretion of pancreatic enzymes, which is in conformity with the theory of exocytosis (47), has been accepted for a long time. Recently, however, a non-parallel enzyme secretion has been reported (15, 81). Furthermore, long-term (2, 40) as well as short-term (16) oral trypsin inhibitor feeding to rats have been shown to induce a non-parallel increase of digestive enzyme activities within the pancreatic tissue. In the present series of experiments the effects of duodenal re-introduction of drained bile-pancreatic juice on the output of individual pancreatic enzymes, i.e. amylase, lipase, and trypsinogen, were recorded in the rat (I). A pronounced and apparently parallel inhibition of the release of each of the three enzymes was observed within one hour. After addition of trypsin inhibitor to the infusate the inhibitory effect on the output of protein and of the different enzymes studied was reversed in a parallel manner reaching levels comparable to those measured before the infusion of bile-pancreatic juice (Fig. 7) (I). Thus, the adaptation of the pancreatic enzyme content to chronic or short-term feeding of trypsin inhibitors was not corresponded by similar changes of the enzyme secretion in acute experiments in fistula rats.

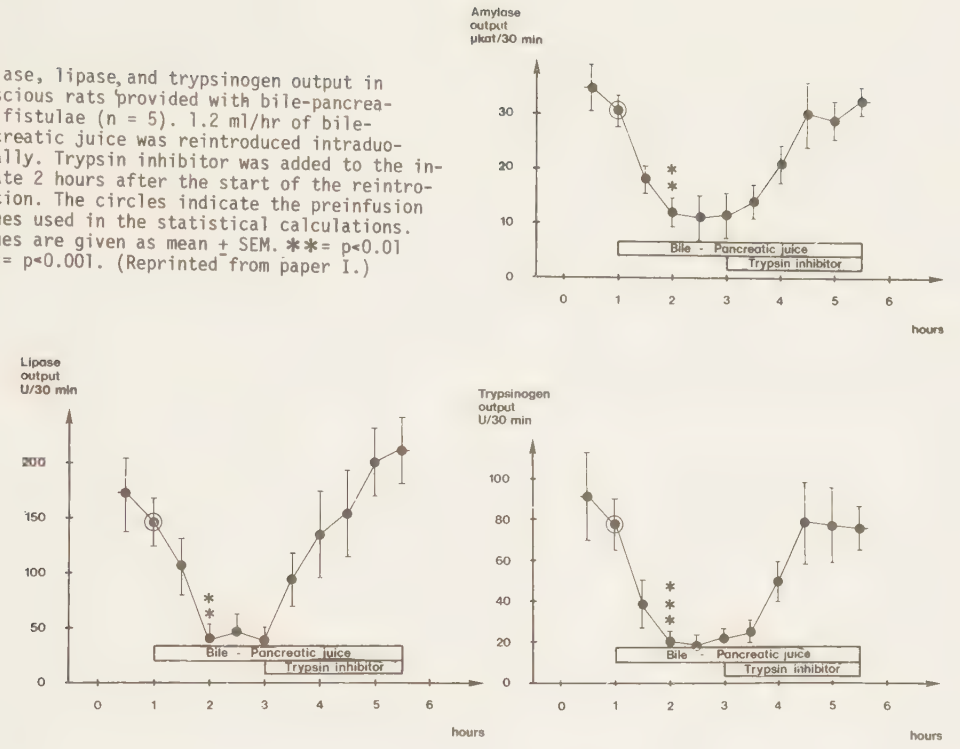


Fig. 7 Amylase, lipase, and trypsinogen output in conscious rats provided with bile-pancreatic fistulae (n = 5). 1.2 ml/hr of bile-pancreatic juice was reintroduced intraduodenally. Trypsin inhibitor was added to the infusate 2 hours after the start of the reintroduction. The circles indicate the preinfusion values used in the statistical calculations. Values are given as mean + SEM. ** = p<0.01 *** = p<0.001. (Reprinted from paper I.)

G. INFLUENCE ON PANCREATIC ENZYME SECRETION OF INTRADUODENAL AMYLASE, LIPASE, AND TRYPSIN

It was demonstrated in the present series of experiments that intraduodenal trypsin infusion in contrast to amylase and lipase depressed pancreatic enzyme secretion. In rat, trypsin affected both secretory volume, protein concentration and trypsin(ogen) activity, while in pig and man mainly the secretory volume was influenced by trypsin. Addition of trypsin inhibitor to the trypsin infusion reversed the inhibitory effects. Trypsin inhibitor per se was without significant effects on the pancreatic secretion.

There is an increasing support of the hypothesis that the secretion of digestive enzymes is a highly regulated process, in which the enzyme content of the pancreatic secretion varies with the composition of food (13, 48). Furthermore, also intraduodenal pancreatic enzyme activity - especially the protease activity - seems to have regulatory effects on the pancreatic secretion (I, II, IV, V, 28). In order to elucidate the effects of intraluminal pancreatic digestive enzymes on pancreatic secretion, amylase, lipase, and trypsin were infused into the duodenum and (bile-)pan-

creatic secretion collected and analysed (I, II, IV, V). Both bacterial (II) and porcine (IV) amylase were administered in amounts calculated to be equivalent to doses of trypsin known to induce inhibition of pancreatic secretion (vide infra). Infusion of amylase to rat (II) and pig (IV) did not influence the protein output, amylase output or the volume of the (bile-)pancreatic secretion. These findings do not agree with those of Macri et al. (58), who reported a relationship between intestinal amylolytic activity and pancreatic morphology and enzyme content. They found that continuous intake of albumin amylase inhibitor by chicken depressed growth rate and caused hypertrophy and increased pancreatic amylase content. They, however, used an amylase inhibitor extracted from wheat flour, which by others (69) has been reported to contain protein components active in inhibiting trypsin as well. Thus, the results reported by Macri et al. may partly be explained by inhibition of intraduodenal trypsin activity.

As it has been demonstrated that the amount of pancreatic lipase secreted adapts itself to the dietary lipid content (71), the question arose whether intraduodenal lipase itself had a significant influence on pancreatic enzyme secretion in the rat (II). Porcine lipase free from co-lipase activity and digestive enzymes was infused into the duodenum in fistula rats. The dose given was equivalent to one of trypsin which was known to depress pancreatic secretion significantly (vide infra). It is well established, that purified pancreatic lipase is inhibited by conjugated bile salts in concentrations above their critical micellar concentration (5). However, as bile was deviated throughout the experiments the lipolytic activity was calculated to be unchanged when the infusion reached the intestinal lumen. In the present experiments in rat, duodenal lipase was found to be without effects on the secretory volume and protein and lipase release (II).

In the absence of intraluminal (bile-)pancreatic juice trypsin infusion into the duodenum markedly depressed pancreatic enzyme output in rat (I, II, III), pig (IV) and man (V)(Fig. 8). In pig and man, the secretory volume was more affected than the activities of the pancreatic enzymes, while in rat volume and enzyme activities decreased roughly in a parallel fashion. Addition of trypsin inhibitor to the infusate resulted in an immediate increase of pancreatic secretion up to a level comparable to that found prior to the trypsin infusion in all species studied. To elucidate if trypsin inhibitor per se had a secretagogue effect, bovine lung trypsin inhibitor was infused into the duodenum of the rat. No effect on the secretion was noted, however (I). Different doses of trypsin (2.5 mg/ml, 5 mg/ml, and 10 mg/ml) were administered, and irrespective of the dose used the inhibitory effect was similar (I, II, III). These findings are in contrast to those reported by Schneeman and Lyman (73), who found a more pronounced depression by 10 mg/ml of trypsin infused as compared to 5 mg/ml. These discrepancies may be due to differences in rate of administration but may also reflect different enzyme activity per unit of weight of the preparations used. Shaw and Heath (74) infused trypsin into the duodenum in pancreatic fistula rats at a concentration of 15 mg/h (equivalent to 8 mg/ml) but were unable to demonstrate changes in pancreatic secretion. The findings of the present

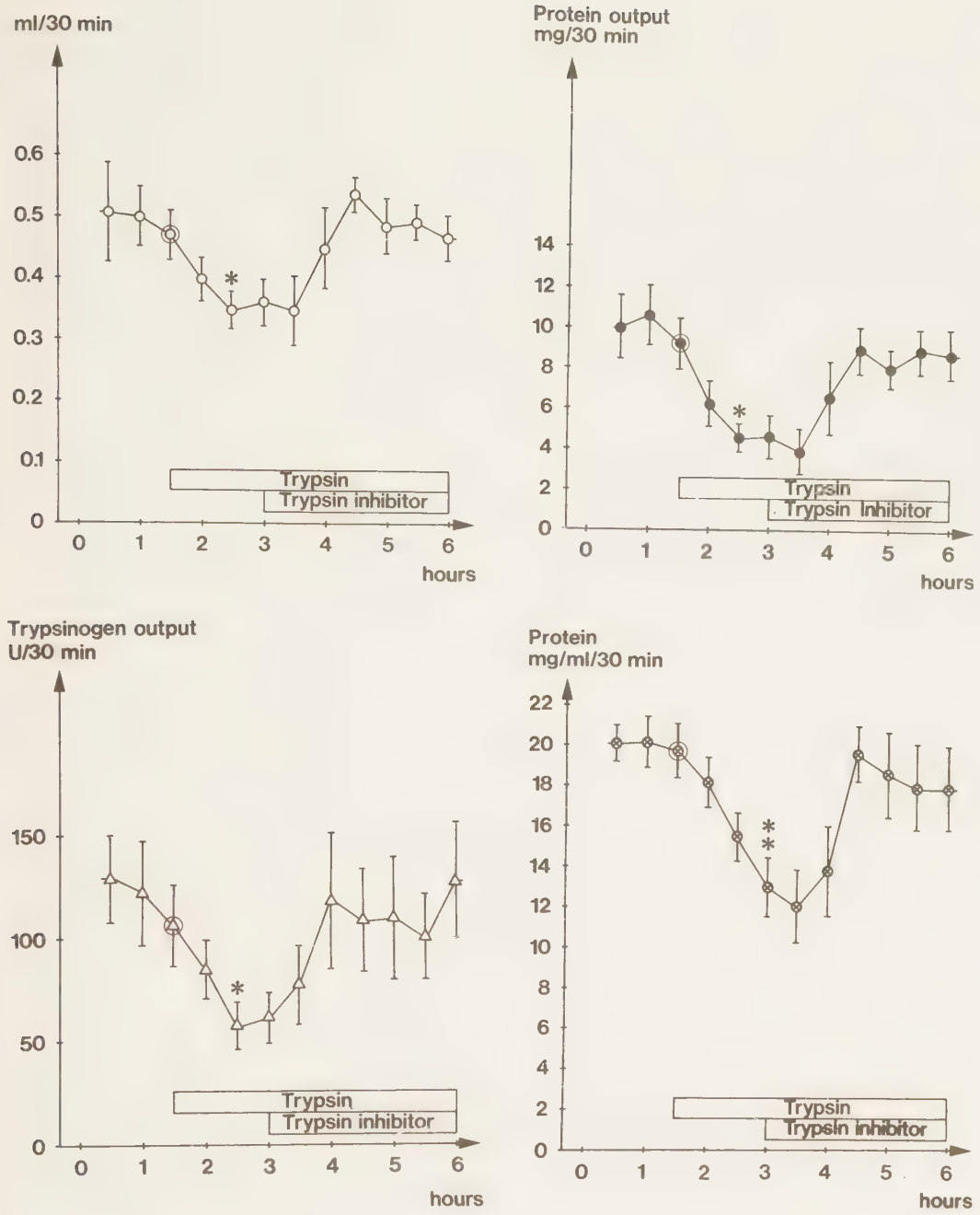


Fig. 8 Influence on secretory volume, protein and trypsinogen output, and protein concentration of intraduodenal infusion of trypsin (2.5 mg/ml 0.05 M $NaHCO_3$) without and with the addition of trypsin inhibitor in conscious rats provided with bile-pancreatic fistulae (n = 5). Values are given as mean ± SEM. The circles indicate the preinfusion values used in the statistical calculations.
*= $p < 0.05$ **= $p < 0.01$ (Reprinted from paper II.)

investigation are compatible with those of Green and Lyman (28) of markedly inhibited pancreatic enzyme secretion by intestinal trypsin in the rat. Green and Lyman, however, did not observe any significant changes in the secretory volume but only in the protein concentration of the (bile-) pancreatic juice secreted. The findings of the present study furthermore support the suggestion made by Corring (12) that intraduodenal trypsin inhibits pancreatic secretion in the pig. Studies by Goldberg et al. (27) on the recovery of bovine trypsin administered orally to patients on ileal drainage are in line with the findings of the present investigation that intestinal trypsin regulates pancreatic enzyme secretion in man (V). They found a lower recovery than expected of the orally given trypsin indicating a suppression of the endogenous trypsinogen secretion. In the present experiments it was demonstrated in a more direct way that trypsin within the duodenal lumen depressed pancreatic enzyme secretion also in man (V).

In summary, intraduodenal trypsin, in contrast to amylase and lipase, was found to exert a feedback regulation of pancreatic enzyme secretion. Lyman et al. (56) suggested that intraduodenal chymotrypsin in contrast to chymotrypsinogen has got similar effects. If other zymogens or pancreatic proteases also are involved in the feedback regulation remains to be elucidated.

H. INFLUENCE ON PANCREATIC ENZYME SECRETION OF INTRADUODENAL BILE

Intraduodenal administration of bile did not influence pancreatic enzyme secretion in rats provided with bile-pancreatic fistulae. Furthermore, bile did not interfere with the depression of pancreatic secretion caused by intraluminal trypsin.

Previous animal experiments have given contradictory results concerning the effect of bile on the exocrine pancreatic secretion. This may be due to differences in experimental design, species, and type of bile preparation used. Mellanby (62) showed that the introduction of bile into the duodenum in cats stimulated the pancreatic secretion. Ivy and Lueth (46) noted that bile was only a weak stimulant to the pancreas of the dog, while Thomas and Crider (78) could not find any stimulating effect of bile in the same species. Staub and Sarles (77) from studies in rat concluded that bile within the duodenal lumen inhibited pancreatic secretion. In man, bile infusion into the duodenum has been demonstrated to have a stimulatory effect on the pancreatic secretion of water, bicarbonate, amylase, and protein (23, 65). After long-term administration of chenodeoxycholic acid to mice O'Donnell et al. (18) reported changes in pancreatic and intestinal enzyme activities similar to those seen after

feeding of trypsin inhibitors, i.e. increased enzyme activities in pancreatic tissue and intestinal content.

From studies in rat (I), pig (IV), and man (V) it was found the reintroduction of (bile-)pancreatic juice into the duodenum markedly inhibited pancreatic enzyme secretion, an effect which was shown to be due chiefly to the tryptic activity of the infusates. Whether bile interferes with the trypsin-induced effects on pancreatic secretion is not known. Therefore, in another experiment pure rat bile free from pancreatic enzyme activity was administered intraduodenally and was found not to influence on secretory volume or protein and amylase output in rats with deviated bile-pancreatic juice flow (stimulated pancreatic secretion) (II). Furthermore, in the presence of intraluminal trypsin (inhibited pancreatic secretion) bile was without effect on pancreatic secretion (Fig. 9) (II). Thus, at least in the rat, bile within the duodenal lumen seemed not to interfere with the inhibitory effects on pancreatic enzyme secretion exerted by trypsin. Again, bile itself did not influence pancreatic enzyme secretion in bile-pancreatic fistula rats.

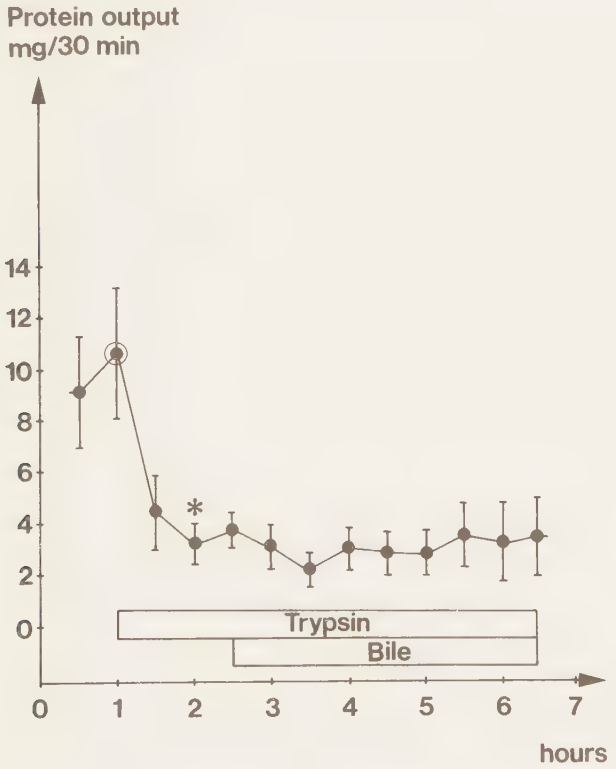


Fig. 9 Effect on protein output of intraduodenal infusion of trypsin and bile in conscious rats provided with bile-pancreatic fistulae (n = 5). Values are given as mean ± SEM. The circle indicates the preinfusion value used in the statistical calculation. * = $p < 0.05$ (Reprinted from paper II.)

I. STUDIES ON POSSIBLE MECHANISMS OF THE TRYPSIN-INDUCED EFFECTS ON PANCREATIC ENZYME SECRETION

Previous studies on trypsin-induced effects on pancreatic enzyme secretion have almost exclusively been concerned with possible hormonal implications. In the present investigation vagal influences on the pancreatic response to intraluminal trypsin were studied. Cholinergic blockade or vagotomy per se resulted in a decreased protein output reflecting both a diminished protein concentration and a reduction in secretory volume in rats provided with bile-pancreatic duct fistulae. Furthermore, contrary to the response in control rats, intraduodenal trypsin infusion did not change the protein output after vagotomy or cholinergic blockade. Vagal stimulation induced by insulin hypoglycaemia failed further to stimulate the enhanced protein output seen in fistula rats. In these latter animals, however, intraduodenal infusion of trypsin markedly inhibited protein secretion. The results suggest that vagal integrity -- at least in acute experiments - is important for the feedback regulation of pancreatic enzyme secretion by intraluminal trypsin.

Two main hypotheses have been produced to explain the trypsin-induced effects on pancreatic enzyme secretion. Green and Lyman (28) suggested that the stimulatory effect of oral trypsin inhibitors on pancreatic secretion was achieved by binding or neutralizing intraluminal trypsin, thereby releasing humoral factor(s) and increasing pancreatic enzyme output. Another hypothesis was presented by Laporte and Trémolière (50) who proposed that trypsin passes the duodenal wall binding CCK-PZ in the blood, thus preventing its stimulation of the pancreas. Consequently, trypsin inhibitors absorbed from the intestine would stimulate pancreatic secretion by eliminating the active trypsin from the blood.

The presence of trypsin, or (bile-)pancreatic juice, within the intestine was found to have influences on pancreatic enzyme secretion in rat (I, II, III), pig (IV) and man (V). It is known that in most species CCK-PZ is released from the upper part of the small intestine and that the distribution of CCK-PZ along the gut parallels that of secretin (7). In the rat it has been demonstrated that resection of the proximal third of the small intestine abolished the effects of long-term administration of trypsin inhibitors on the pancreas (36). Furthermore, parenteral trypsin inhibitor administration did not result in changes of pancreatic morphology or enzyme activity (39). In other experiments treatment of rats with CCK-PZ or the octapeptide of CCK-PZ or cerulein plus secretin resulted in changes similar to those seen after long-term oral trypsin inhibitor treatment (26, 42, 68). Recently it was found that oral ingestion of trypsin inhibitors altered bile composition but not bile flow in the rat (51). Also changes in glucose tolerance, insulin secretion and glucose oxidation in peripheral tissues have been reported after trypsin inhibitor feeding to the rat (39, 40, 54). Schneeman et al. (73) in acute experiments found that infusion of trypsin into the distal part

of the intestine was without significance to the pancreatic enzyme secretion. They concluded that a feedback control by trypsin operates in the area of the gut from which hormones affecting pancreatic cells are released. The findings reported are consistent with the concept that trypsin in the upper small intestine prevents the release of a hormone/hormones into the blood, and that trypsin inhibitors by binding trypsin effectively removes the enzyme from the intestine, thereby releasing a hormone/hormones and increasing pancreatic enzyme output. A more direct evidence for this hypothesis is provided by the report by Khayambashi and Lyman (49) who showed that plasma from rats fed soy bean trypsin inhibitor stimulated amylase secretion in the isolated perfused rat pancreas. Further support for this hypothesis is the report by Toregard et al. (79), who used pancreatic isolated cells in suspension and found that plasma from rats fed soy bean trypsin inhibitor caused increased lipase and amylase secretion from the cells. In contrast, Lyman et al. (57) in an early study were unable to demonstrate the release of a humoral factor/factors by trypsin inhibitor administration in cross-circulation experiments in the rat.

The data referred to above are in accordance with an intraluminal mode of action of trypsin and trypsin inhibitors, respectively. This hypothesis is further supported by the considerable variation in the molecular size and structure of different trypsin inhibitors (25, 45), which induce effects on the pancreas. This makes it rather unlikely that there is an interaction between a specific cell surface receptor and trypsin inhibitor either in the intestinal mucosa or in the pancreas. The need for active trypsin to be present in the digestive tract in order to suppress digestive enzyme secretion was also indicated by the findings of Schneeman et al. (73), that trypsin bound to diisopropylphosphorofluoridate (DFP-trypsin) did not suppress enzyme secretion as did the active enzyme. Lyman et al. (56) have previously reported that the inactive precursor of chymotrypsin (chymotrypsinogen) also did not suppress enzyme secretion in the rat as did active chymotrypsin. The active centre of the molecule is blocked either by DFP as with trypsin or by a peptidergic structure as seems to be the case in chymotrypsinogen. Thus it seems as if trypsin or chymotrypsin are required in active form in order to control the feedback system.

The proposed theory by Götze and Rothman (31) of an entero-pancreatic circulation of digestive enzymes may speak in favour of the hypothesis of Laporte and Trémolière of a direct passage of trypsin into the blood stream acting on the pancreatic acinar cell. On the other hand Borgström et al. (4) using radioimmunological technique were unable to demonstrate trypsin in plasma. Recently Wilson et al. (82) found immunocytochemical evidence that soy bean trypsin inhibitor does not enter the cells of the intestinal mucosa in the adult rat. Further arguments against the hypothesis of absorption of trypsin or trypsin inhibitor are the findings of Schneeman et al. (73), that intravenous infusion of trypsin or trypsin inhibitors to rat was without effect on pancreatic enzyme secretion. In the present investigation it was found that intraduodenal infusion of trypsin inhibitor in the absence of intestinal pancreatic juice or intestinal trypsin in fistula rats was without effect on the pancreatic

secretion (I). This finding also supports the idea that active trypsin within the duodenal lumen is essential for the stimulation of pancreatic secretion by trypsin inhibitor. Considering previous reports and the results of the present experiments, it seems reasonable to assume a hormonal mediation of the observed feedback regulation of pancreatic digestive enzymes.

Most authors have suggested that peptides of the CCK group are responsible for this enzyme response. Recently Peterson and Grossman reported that also bicarbonate secretion was affected by intraduodenal bile-pancreatic juice in rat (67). Furthermore the results of the present experiments in pig show that intraluminal trypsin influences pancreatic secretory volume as well (IV). Therefore hormones such as secretin should also be considered as possible mediators.

Practically no attention has been paid to the possible role of neural influence in this context. In a study by Lyman et al. (57) it was concluded that the pancreatic response to long-term administration of trypsin inhibitors seemed not to be dependent upon cholinergic nerve pathways. Furthermore, Grossman (30) and Petersen and Grossman (67) did not find any evidence of vagal influences on the pancreatic secretory response to diversion of bile-pancreatic juice from the intestine. These results are in contrast to the findings of the present study of decreased protein output in fistula rats subjected to controlled vagotomy or cholinergic blockade in two different experiments (III). Furthermore, the findings of this study suggest that vagal integrity is of importance for the feedback regulation of pancreatic secretion exerted by intraluminal trypsin, as the trypsin-induced changes of the protein output in fistula rats was abolished by vagotomy as well as by cholinergic blockade (Fig. 10) (III). Indirect vagal stimulation by insulin-induced hypoglycaemia did not bring about any further increase of the pancreatic protein release in rats provided with bile-pancreatic fistulae. In these rats, however, intraduodenal trypsin infusion depressed the protein output, although the response was slower in onset and more gradual than in the control rats (III).

The results of the present study point to an interaction between the autonomic nervous system and the gastrointestinal hormones on pancreatic protein secretion. Most data published favour a hormonal mediation of the feedback mechanism studied. However, considering the present results of the vagotomy and cholinergic blockade experiments, it is obvious that vagal integrity, at least in acute experiments, is also important. Thus feedback effects on pancreatic enzyme secretion exerted by intraluminal trypsin in the rat seem to be regulated by a complex interaction between hormonal and neural stimuli.

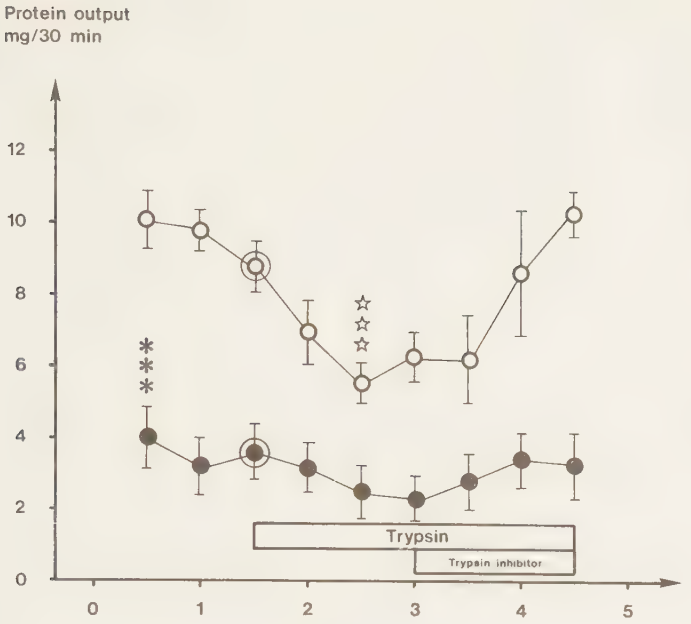


Fig. 10 Protein output in vagotomized rats (●—●; n = 9) and in control rats (○—○; n = 8) provided with bile-pancreatic fistulae. Trypsin (5 mg/ml 0.05 M $NaHCO_3$) was administered intraduodenally either without or with the addition of trypsin inhibitor. Values are given as mean ± SEM. The circles represent pre-infusion values. Probability levels of significant differences are indicated by (*) for differences between groups and by (☆) for differences related to preinfusion levels within the same group.
*** = $p < 0.001$ ☆☆☆ = $p < 0.001$ (Reprinted from paper III.)

J. INTESTINAL PANCREATIC ENZYME ACTIVITIES FOLLOWING ENZYME SUBSTITUTION THERAPY

In vitro testing of four commercially available pancreatin preparations with different galenic properties showed that the enzyme activities varied widely with the greatest variations concerning lipase and trypsin. Pancreatin in the form of tablets, with or without protective devices against acid, did not cause any apparent increase of the activities of pancreatic enzymes in the upper part of the gut in patients with pancreatic insufficiency. Granulated pancreatin, on the other hand, brought about an increase of the activities of amylase, lipase, phospholipase, and trypsin. A proportionally greater amount of active enzymes reached the small intestine when administered as granulated pancreatin as compared to tablets.

Pancreatic supplements, which mainly are composed of lyophilized porcine pancreatic glands, may have the potential of increasing intraduodenal pancreatic digestive enzyme activities and therefore, according to the results of the present study, may be able to interfere with exocrine pancreatic function. This suggestion is supported by findings of Trémolière

(80), who noted a cessation of the flow of pancreatic juice after oral ingestion of high doses of lyophilized pancreas in patients with pancreatic fistulae. On the other hand, DiMagno et al. (17) in a small number of patients observed, that only 22 % of trypsin and 8 % of lipase administered reached the ligament of Treitz in active form. After pancreatin administration to patients with advanced pancreatic insufficiency the intestinal activity of trypsin at its most reached 3 % and lipase 1 % of the mean activities in healthy subjects. Therefore, a study of the effect of oral administration of four galenically different preparations of pancreatin on intestinal activities of amylase, lipase, phospholipase and trypsin was performed (VI). Additionally, the relation between *in vitro* and *in vivo* activities of the different preparations was examined (VI).

The *in vitro* analyses, in which human small intestinal aspirates were used as incubation medium, demonstrated high but varying enzyme activities of the four pancreatic supplements (VI, 43). In the present study human small intestinal juice was used as the incubation medium in order to imitate physiological conditions as close as possible. Thus, the pancreatic extracts were incubated in a milieu containing naturally occurring activators and inhibitors of pancreatic enzymes and bile acids. Another reason why human duodenal juice was chosen was that it may give a more reliable comparison between *in vitro* activities and *in vivo* activities (human small intestinal juice aspirated after oral administration of pancreatic extracts).

Indirect stimulation of the pancreas by means of a test meal as described by Lundh (53) has been found to be a valuable test of pancreatic function and digestive capacity in man (38). It was found to be suitable for an evaluation of the ability of pancreatic substitution preparations to affect the enzyme activities in the upper part of the small intestine (VI). Pancreatin in the form of a simple tablet (Pancreozym®), when given to healthy persons in twice the recommended dose, did not cause significant changes of either amylase, lipase, phospholipase or trypsin within the duodenal lumen (VI). Furthermore, neither this preparation nor pancreatin in the form of a tablet with tannin-binding as protection against acid (Pancreon forte®) did significantly affect the intestinal enzyme activities in patients suffering from pancreatic insufficiency (VI). The two preparations in granulated form, however, both with protective properties against acid (Pancreon granules®; Pancreatin granules®), brought about an increase of the activities of amylase, phospholipase, and trypsin, which was most pronounced during the second hour after administration (Fig. 11). The lipase activities, however, were less affected as compared to those of the other enzymes studied, although a slight elevation was found in a few intestinal juice aliquots (VI).

The reason why the lipase activities (Fig. 11) differ from those of the other enzymes is not fully understood. They might reflect an increased vulnerability of this enzyme to gastric juice (32). However, the buffering capacity of the test meal caused a rise in pH of the stomach content (Fig. 12) which should allow lipase to pass through the stomach at least during the first 30 minutes after the administration. Further, Reagan et al (70) and Saunders et al. (72) found almost normal amounts of enzyme

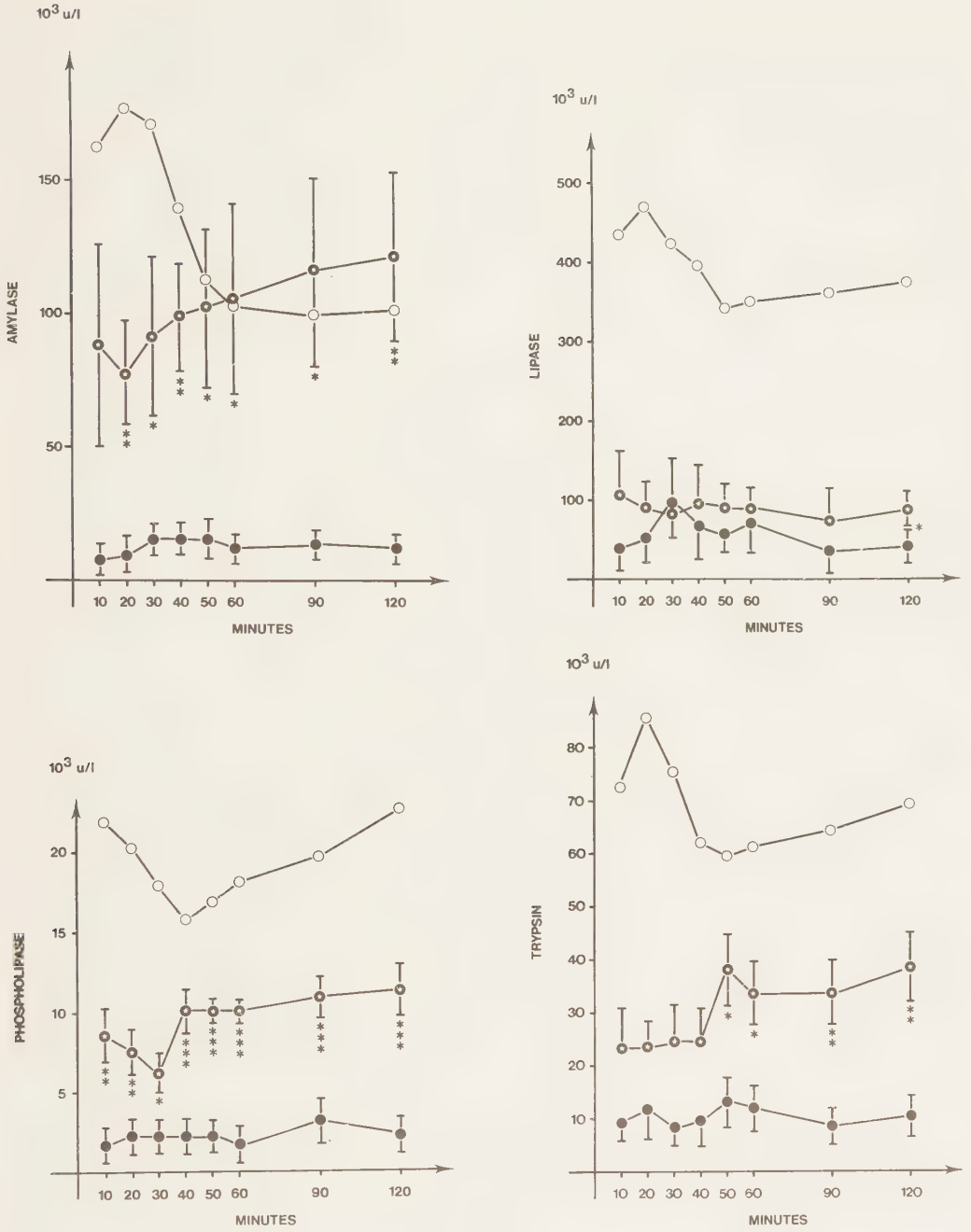


Fig. 11 Intestinal amylase, lipase, phospholipase and trypsin activities in patients with pancreatic insufficiency (n = 6) with (⊛—⊛) and without (●—●) a granulated pancreatin supplementation. Open circles (○—○) represent normal subjects (reference 44). Amylase activity is expressed as µ mol maltose released per ml per min. Lipase activity is expressed as µ mol fatty acids released per ml per min. Phospholipase activity is expressed as µ mol fatty acids released per ml per min. Trypsin activity is expressed as µ mol TAME hydrolyzed per ml per min. Values are given as mean ± SEM.
* = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.001$

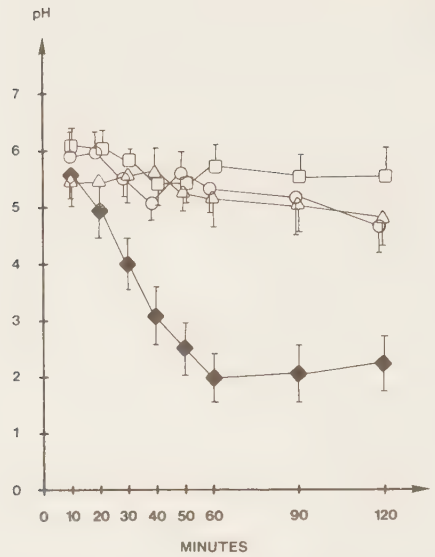


Fig. 12 pH in aspirates from the stomach and the intestine, respectively sampled during 2 hours following a test meal. Values are given as mean + SEM.
○ = duodenal pH in chronic pancreatitis (n = 10)
△ = duodenal pH in unoperated pancreatic cancer (n = 8)
□ = intestinal pH in operated pancreatic cancer (total pancreatectomy) (n = 6)
◆ = pH in the stomach in pancreatic insufficiency (n = 6)

activity in the duodenum after administration of pancreatin together with methiamide and antacids to patients with pancreatic insufficiency, except that the amount of lipase was considerably lower than normal. Therefore it seemed as if inactivation of lipase by gastric juice was not the sole mechanism involved. In this context, it should be noted that no differences in pH of the intestinal aspirates were found between the patient groups in the present study (Fig. 12) (VI). Another explanation of the different lipase curves obtained after supplementation therapy could be inactivation of the enzyme during collection and storage of the intestinal juice, However, in a previous study on healthy subjects (44) identical activity curves of amylase, lipase, phospholipase, and trypsin were observed in intestinal aspirates collected during two hours after ingestion of a test meal.

A comparison between pancreatic enzyme activities in the test meal given and in the intestinal aspirate suggested that proportionally greater amounts of active enzymes administered as granules reached the duodenum (VI). The delivery of enzymes into the duodenum started after approximately 40 minutes and was most pronounced during the second post-prandial hour (VI). Reagan et al. (70) and diMagno et al. (17) noted peak inflow of enzymes into the duodenum one hour after ingestion of the pancreatin preparation. Malagelada et al. (60) recently reported that the greatest intragastric activity occurred within one hour after eating, when homogenization, dilution and acidification of gastric contents took place, and Meyer et al. (63) in studies on gastric emptying of solid materials showed that the post-cibal stomach retained non-dispersed

solid material of more than a few mm in size. These observations are in accordance with the better digestive capacity found after administration of granulated preparations in the present study and, further, might partly explain the late delivery of enzymes into the duodenum.

Considering the findings reported above pancreatic substitution preparations in granulated form cause a marked increase of intestinal enzyme activities in patients with pancreatic insufficiency and therefore may interfere with the observed feedback regulation of pancreatic enzyme secretion.

K. FEEDBACK REGULATION OF PANCREATIC ENZYME SECRETION THROUGH VARIATIONS OF INTRALUMINAL TRYPSIN; POSSIBLE CLINICAL IMPLICATIONS

As shown in the present thesis, intraduodenal tryptic activity influences pancreatic enzyme secretion in the rat (I, II, III), in the pig (IV), and in man (V). Trypsin within the duodenal lumen inhibits pancreatic secretion possibly by interfering with the release of humoral factor(s) from the duodenal mucosa operating on the pancreatic acinar cell. In the absence of intraluminal trypsin pancreatic secretion is stimulated, which may be due to the release of a gastrointestinal hormone/hormones from the duodenal wall. Such a regulatory mechanism may have clinical implications in situations, in which it is desirable to manipulate with the stimulation of the exocrine pancreas. One of the main principles in the treatment of acute pancreatitis is to reduce the activity of the gland by diminishing the neural and hormonal stimulation of it (66, 64). This is generally aimed at by fasting, gastric intubation and administration of anticholinergic agents and gastrointestinal hormones known to inhibit pancreatic secretion (e.g. glucagon and somatostatin). The results of the present study show that a suppression of pancreatic function can be attained also by influencing the feedback regulation. Therefore it would be worthwhile to test the effect of intraduodenal trypsin infusion on the course of acute pancreatitis. In chronic pancreatitis, suppression of the stimulation of the pancreas by increased intraduodenal tryptic activity theoretically would diminish the intraductal pressure behind the strictures in the pancreatic ducts and thereby give relief from pain. In this respect some enzyme preparations are preferable to others because of higher enzyme activities (VI, 43). Furthermore, the results suggest that pancreatin preparations in granulated form in contrast to tablets cause proportionally higher tryptic activities within the small intestine (VI) and therefore may be able to interfere with the observed feedback regulation of pancreatic secretion. Finally, the healing of pancreatic fistulae can be supposed to be accelerated by intraduodenal trypsin infusion via inhibition of the pancreatic secretion.

L. SUMMARY

This thesis deals with the influence of bile-pancreatic juice components on pancreatic enzyme secretion in rat, pig and man. In all three species deviation of (bile-)pancreatic juice from the duodenum resulted in hypersecretion of pancreatic enzymes. This hypersecretion was reversed by re-infusion of (bile-)pancreatic juice. Intraduodenal administration of trypsin inhibitor in the presence of (bile-)pancreatic juice enhanced the pancreatic enzyme secretion. In the rat these secretory changes were characterized by a parallel increase as well as a parallel decrease of the output of amylase, lipase and trypsinogen. Trypsin inhibitor per se was without effect. Intraduodenal infusion of amylase (rat and pig) or lipase (rat) did not influence pancreatic enzyme secretion, which, however, was markedly depressed by trypsin infusion (all species). Intraduodenal administration of bile in the absence of intraluminal trypsin (stimulated pancreatic secretion) was without effect on the pancreatic enzyme secretion in the rat. Furthermore, bile infusion did not affect the inhibitory action on pancreatic enzyme secretion exerted by intraluminal trypsin. The results obtained support and extend previous studies suggesting that pancreatic enzyme secretion is regulated via a feedback mechanism controlled by intraluminal trypsin.

Vagotomy or cholinergic blockade resulted in a decreased protein output in rats provided with bile-pancreatic duct fistulae. Intraduodenal administration of trypsin did not change the protein output after vagotomy or cholinergic blockade. Vagal stimulation induced by insulin hypoglycaemia failed further to enhance the protein output in fistula rats. Intraduodenal infusion of trypsin during insulin-induced hypoglycaemia, however, markedly inhibited protein secretion. The results indicate that intact vagal function is essential for the feedback regulation of pancreatic enzyme secretion exerted by intraluminal trypsin.

Pancreatic supplements did not cause any apparent increase in the activities of pancreatic enzymes in the upper part of the gut when administered as tablets to healthy subjects or to patients with exocrine pancreatic insufficiency. Granulated pancreatin, on the other hand, brought about an increase of the activities of amylase, lipase, phospholipase, and trypsin in patients with pancreatic insufficiency. Thus, granulated pancreatin may induce changes of intestinal enzyme activity great enough to interfere with the observed feedback mechanism.

The postulated feedback regulation of exocrine pancreatic secretion may have clinical and therapeutic implications in pancreatic disease. It presents a challenge for studies on the value of intraduodenal administration of trypsin in acute pancreatitis in order to reduce the activity of the pancreatic gland. Furthermore, in chronic pancreatitis, suppression of the hormonal stimulation of the pancreas by effective substitution therapy may diminish the intraductal pressure behind strictures in the pancreatic ducts and give relief from pain. Finally, the healing of pancreatic fistulae would be facilitated by intraduodenal trypsin administration.

M. ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to:

Professor Stig Bengmark, my chief, for never failing encouragement.

Associate Professor Ingemar Ihse, my teacher, for the initiation of the study and for all his advice, invaluable help, and guidance throughout this work.

Associate Professor Ingmar Lundquist, my teacher, for enthusiastic guidance and invaluable help.

Dr Anders Evander for fruitful discussions.

Mrs' Birgit Persson, Elisabeth Pollak, Birgit Andersson and Monica Radnell for skilful technical assistance, and Miss Eva Kroon, Miss Eva Edestad and Miss Agneta Berglund for skilful secreterial and illustrative assistance.

Mr Ove Bengtsson, who helped with the printing.

Dr Gunnar Isaksson, who corrected the English text.

The investigation was supported by grants from Swedish Medical Research Council (Projects Number 17X-03012; 04P-4289), The Medical Faculty of Lund, Albert Påhlsson Foundation and Greta and Harald Jeansson's Foundation.

N. REFERENCES

1. Annis, D. & Hallenbeck, G.A.
Proc. Soc. Exp. Biol. 1951, 77, 383.

2. Arnesjö, B., Ihse, I., Lundquist, I. & Quist, I.
Scand. J. Gastroent. 1973, 8, 545.

3. Babkin, B.P.
Zentralbl. Gestamte Physiol. Pathol. Stoffwechsels 1906, 1, 98.

4. Borgström, A. & Ohlsson, K.
Scand. J. Clin. Lab. Invest. 1976, 36, 809.

5. Borgström, B. & Erlanson, C.
Biochim. Biophys. Acta 1971, 242, 509.

6. Brooks, F.P.
Am. J. Clin. Nutr. 1973, 26, 291.

7. Bryant, M.G. & Bloom, S.R.
Gut 1979, 20, 653.

8. Ceska, M., Birath, K. & Brown, B.
Clin. Chim. Acta 1969, 26, 437.

9. Chernick, S.S., Lepkowsky, S. & Chaikoff, I.L.
Am. J. Physiol. 1948, 155, 33.

10. Cook, A.R., Nahrwold, D.L. & Grossman, M.I.
Am. J. Physiol. 1967, 213, 637.

11. Corring, T.
Ann. Biol. Anim. Biochim. Biophys. 1973, 13, 755.

12. Corring, T.
Ann. Biol. Anim. Biochim. Biophys. 1974, 14, 487.

13. Corring, T.
World Rev. Nutr. Diet. 1977, 27, 132.

14. Dahlqvist, A.
Scand. J. Clin. Lab. Invest. 1962, 14, 145.

15. Dagorn, J.C. & Mongeau, R.
Biochim.Biophys. Acta 1977, 498, 76.

16. Dijkhof, J. & Poort, C.
Biochim. Biophys. Acta 1978, 543, 167.

17. DiMagno, E.P., Malagelada, J.R., Go, V.L.W. & Moertel, C.G.
N. Engl. J. Med. 1977, 296, 1318.

18. O'Donnell, M.D., Fitzgerald, O. & Mc Geeney, K.F.
Scand. J. Gastroent. 1977, 12, 683.

19. Donnér, J.
Acta Chem. Scand. 1976, B30, 430.

20. Eggstein, M. & Creutz, F.M.
Klin. Wschr. 1955, 33, 879.

21. Erlanson, C. & Borgström, B.
Scand. J. Gastroent. 1970, 5, 293.

22. Evander, A., Ihse, I. & Lundquist, I.
Danish Med. Bull. 1979, 26, suppl. 1, p 13.

23. Forell, M.M., Otte, M., Kohl, H.J. et al.
Scand. J. Gastroent. 1971, 6, 261.

24. Frey, E.K., Kraut, H. & Werle, E.
Das Kallikrein-Kinin System und seine Inhibitoren.
Ferdinand Enke Verlag, Stuttgart, 1967, 126

25. Fritz, H., Hüller, I, Wiedemann, M. & Weile, E.
Hoppe Seylers Z. Physiol. Chem. 1967, 348, 405.

26. Fölsch, U.R., Winckler, K. & Wormsley, K.G.
Scand. J. Gastroent. 1978, 13, 663.

27. Goldberg, D.M., Campbell, R. & Roy, A.D.
Gut 1969, 10, 477.

28. Green, G.M. & Lyman, R.L.
Proc. Soc. Exp. Biol. Med. 1972, 140, 6.

29. Green, G.M., Olds, B.A., Matthews, G. & Lyman, R.L.
Proc. Soc. Exp. Biol. Med. 1973, 142, 1162.

30. Grossman, M.I.
Am. J. Physiol. 1958, 194, 535.

31. Götze, H. & Rothman, S.S.
Nature 1975, 257, 607.

32. Heizer, W.D., Cleaveland, C.R. & Iber, F.J.
John Hopk. Med. J. 1965, 116, 261.

33. Henriksen, F.W.
Dissertation, FADL's Forlag, Copenhagen, 1974

34. Hummel, B.C.W.
Canad. J. Biochem. Biophys. 1959, 37, 1393.

35. Håkanson, R., Alumets, J., Ekelund, M. et al.
Scand. J. Gastroent. 1978, suppl. 55, 24

36. Ihse, I.
Scand. J. Gastroent. 1976, 11, 11

37. Ihse, I. & Arnesjö, B.
Acta Chem. Scand. 1973, 27, 2749.

38. Ihse, I., Arnesjö, B., Lilja, P. & Kugelberg, C.
Scand. J. Gastroent. 1977, 12, 663.

39. Ihse, I., Arnesjö, B. & Lundquist, I.
Scand. J. Gastroent. 1974, 9, 719.

40. Ihse, I., Arnesjö, B. & Lundquist, I.
Scand. J. Gastroent. 1975, 10, 321.

41. Ihse, I., Arnesjö, B. & Lundquist, I.
Scand. J. Gastroent. 1976, 11, 363.

42. Ihse, I., Arnesjö, B. & Lundquist, I.
Scand. J. Gastroent. 1976, 11, 529.

43. Ihse, I. & Lilja, P.
Digestion 1979, 19, 48.

44. Ihse, I., Lilja, P., Evander, A. & Skude, G.
Scand. J. Gastroent. 1977, 12, 629.

45. Ikenaka, T., Odani, S. & Koide, T.
In Bayer Symposium V "Proteinase Inhibitors" (Ed's Fritz, H., Tschesche, H., Greene, L.J. & Truscheit, E.), Springer Verlag, 1974, 325.

46. Ivy, A.C. & Lueth, H.C.
Proc. Soc. Exp. Biol. Med. 1927, 24, 837.

47. Jamiesson, J.O. & Palade, G.E.
J. Cell. Biol. 1971, 48, 503.

48. Johnson, A., Hurwitz, R. & Kretchmer, N.
J. Nutr. 1977, 87,

49. Khayambashi, H. & Lyman, R.L.
Am. J. Physiol. 1969, 217, 646.

50. Laporte, J.C. & Trêmolières.
Nutr. Metabol. 1973, 15, 192.

51. Lilja, P., Nilsson, A. & Ihse, I.
XIII:th Scand. Congr. Gastroent., Espoo, Finland 1979 (Abstract).

52. Lowry, O.H., Rosenbrough, N.J., Farr, A.C. & Randall, R.J.
J. Biol. Chem. 1951, 193, 265.

53. Lundh, G.
Gastroenterology, 1962, 42, 275.

54. Lundquist, I., Ihse, I. & Arnesjö, B.
Scand. J. Gastroent. 1976, 11, 369.

55. Lyman, R.L.
J. Nutr. 1957, 62, 285.

56. Lyman, R.L, Olds, B.A. & Green, G.M.
J. Nutr. 1974, 104, 105.

57. Lyman, R.L., Wilcox, S.S. & Monsen, E.R.
Am. J. Physiol. 1962, 202, 1077.

58. Macri, A., Parlamenti, R., Silano, V. & Valfre, F.
Poultry Science 1977, 56, 434.

59. Magee, D.F. & Hong, S.S.
Swine in Biomedical Research 1965, 109

60. Malagelada, J.R., Longstreth, G.R., Summerskill, W.H.J. & Go, V.L.W.
Gastroenterology 1976, 70, 203.

61. Meldolesi, J.
Pharmacol. Res. Commun. 1976, 1, 1.

62. Mellanby, J.
J. Physiol. 1926, 61, 419.

63. Meyer, J.H.
N. Engl. J. Med. 1977, 296, 1347.

64. Myren, J.
Scand. J. Gastroent. 1977, 12, 513.

65. Osnes, M., Hansen, L.E., Flaten, O. & Myren, J.
Gut 1978, 19, 180.

66. Paxton, J.R. & Payne, J.H.
Surg. Gynecol. Obstet. 1948, 86, 69.

67. Petersen, H. & Grossman, M.J.
Am. J. Physiol. 1977, 233 (6), E530.

68. Petersen, H., Solomon, T. & Grossman, M.J.
Gastroenterology 1979, 76, 790.

69. Petrucci, T., Tomasi, M., Cantagalli, P. & Silano, V.
Phytochemistry 1974, 13, 2487.

70. Reagan, T.P., Malagelada, J.R., DiMagno, E.P. et al.
N. Engl. J. Med. 1977, 297, 854.

71. Robberecht, P., Deschodt-Lanckman, M., Camus, J. & Christophe, J.
Arch. Int. Physiol. Biochim. 1971, 79, 206.

72. Saunders, J.H.B., Drummond, S. & Wormsley, K.G.
Br. Med. J. 1977, 1, 418.

73. Schneeman, B.O. & Lyman, R.L.
Proc. Soc. Exp. Biol. Med. 1975, 148, 879.

74. Shaw, H.M. & Heath, T.J.
Q. J. Exp. Physiol. 1973, 58, 335.

75. Shay, H., Komarov, S.A. & Gruenstein, M.
Arch. Surg. 1949, 59, 210.

76. Singh, M. & Webster, P.D.
Gastroenterology 1978, 74, 294.

77. Staub, J.L. & Sarles, H.
European Pancreatic Club, Dublin, 1978, p 48 (Abstract).

78. Thomas, J.E. & Crider, J.O.
Am. J. Physiol. 1943, 138, 548.

79. Toregard, B.M., Borgström, B., Ihse, I. & Nilsson, A.
Danish Med. Bull. 1979, 26, suppl. 1, p6.

80. Trémolière, J.
Med. Hyg. 1971, 29, 1508.

81. de Waele, B., Lambin, A., de Smul & Kiekens, R.
Digestion 1979, 19, 15.

82. Wilson, P.A., Melmed, R.N., Hampe, M.M.V. & Holt, S.J.
Gut 1978, 19, 260.

PRINTING
ILLUSTRATION DEPARTMENT
DEPARTMENT OF SURGERY
UNIVERSITY OF LUND
1979